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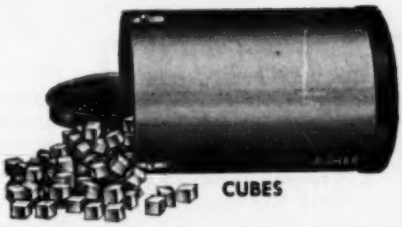

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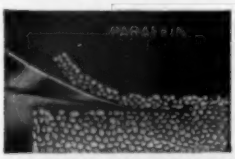

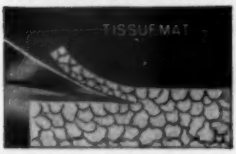

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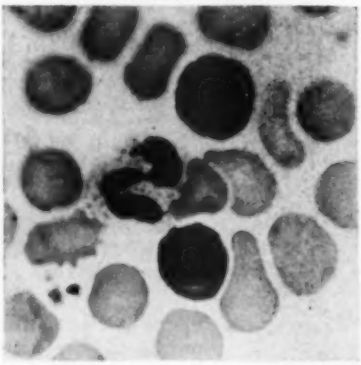

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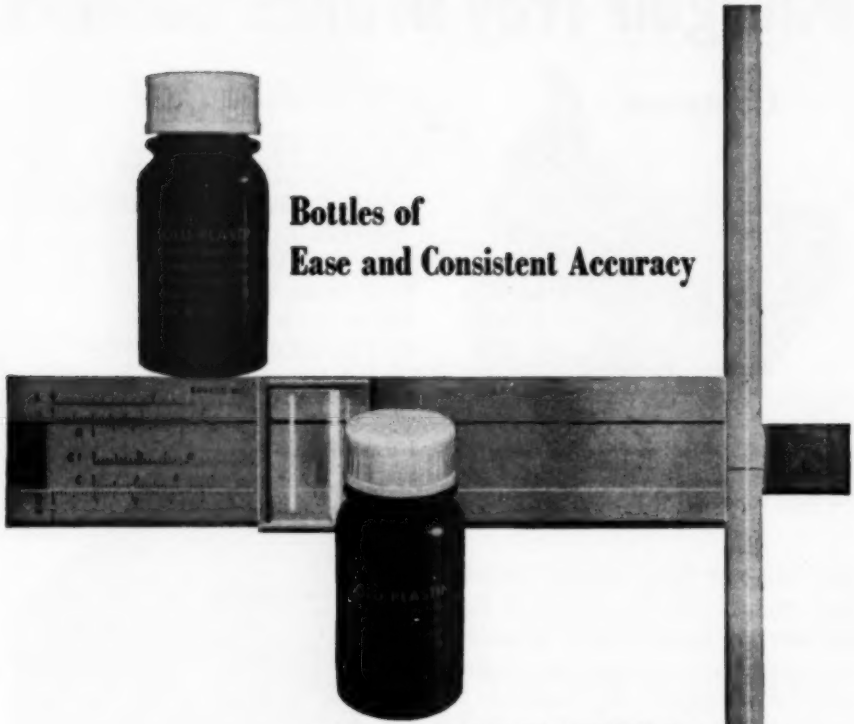

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FORM OF HEPATITIS IN NEONATAL PERIOD SIMULATING BILIARY ATRESIA

JOHN M. CRAIG, M.D.
AND
BENJAMIN H. LANDING, M.D.
BOSTON

INTRODUCTION

SINCE icterus is physiologic in the neonatal period, and, since the jaundice arising from congenital obstruction of the bile ducts often does not appear clinically until the second week of life or later, the correct etiologic diagnosis of jaundice in the newborn is difficult. The chief causes of prolonged neonatal icterus, giving a clinical picture of obstructive jaundice, include biliary atresia in 60% of the cases,[1] the posterythroblastotic state in 15%, and an ill-defined group in the remaining 25%. The latter group includes cases of hepatitis and prolonged jaundice having many of the clinical features of regurgitative jaundice. Our immediate concern is with a number of patients in the latter group who have the onset of severe icterus immediately after birth or in the early neonatal period. In these patients, the stools are acholic, urobilinogen is not present in the urine, results of liver function tests are equivocal, and a normally patent biliary tree is present on abdominal exploration or at postmortem examination. While in a few of these patients liver biopsy discloses absence or a decrease in the number of the intrahepatic bile ducts [2] a larger number of patients appear to have histologically specific hepatitis. Stokes, Wolman, and others [3] described a similar group of cases in 1951. The toxic or bacterial hepatitis of "parenteral" origin due to severe sepsis, omphalitis, diarrhea, pyelonephritis, or peritonitis usually can be excluded on clinical grounds alone. Also, the pathologic changes in the liver in this type of hepatitis usually are distinct.

MATERIALS AND METHODS

The material in this report includes all the surgical liver biopsy specimens in the department of pathology of The Children's Medical Center from January, 1941, to March, 1952, and the autopsy protocols over the same period. From the latter all specimens were selected in which the final diagnosis suggested the presence of significant liver disease. Seventy liver biopsy specimens, 2,500 autopsy protocols, and 103 liver specimens obtained at postmortem examination were reviewed. These tissues had been routinely fixed in Zenker's acetic acid solution, in 10% formalin, and in absolute alcohol. The surgical tissues were stained with eosin-methylene blue

From the Departments of Pathology, Harvard Medical School and The Children's Medical Center, and The Children's Cancer Research Foundation.

1. Hsia, D.; Patterson, P.; Allen, F. H.; Diamond, L. K., and Gellis, S. S.: Pediatrics **10**:243, 1952.

2. Ahrens, E. H., Jr.; Harris, R. C., and MacMahon, H. E.: Pediatrics **8**:628, 1951.

3. Stokes, J., Jr.; Wolman, I. J.; Blanchard, M. C., and Farquhar, J. D.: A. M. A. Am. J. Dis. Child. **82**:213, 1951.

or eosine-toluidine blue and the autopsy tissue with hematoxylin and eosin. In addition, Best's carmine and the periodic acid leucofuchsin stains for glycogen, Sudan 4 or Sudan black stain for lipid, Mallory's aniline blue and phosphotungstic acid stains for connective tissue, Foot's stain for reticulum, and Taft's[4] methyl green-pyronin staining procedures were carried out on a number of the specimens.

The histologic specimens from the autopsy and surgical series were examined without foreknowledge of the clinical course or nature of the disease in the patient from whom the specimen had been obtained. The slides were examined and evaluated independently by each of the authors.

RESULTS

It became apparent immediately that not only was there consistent agreement as to the classification of the specimens, but a group of livers having a distinctive, and

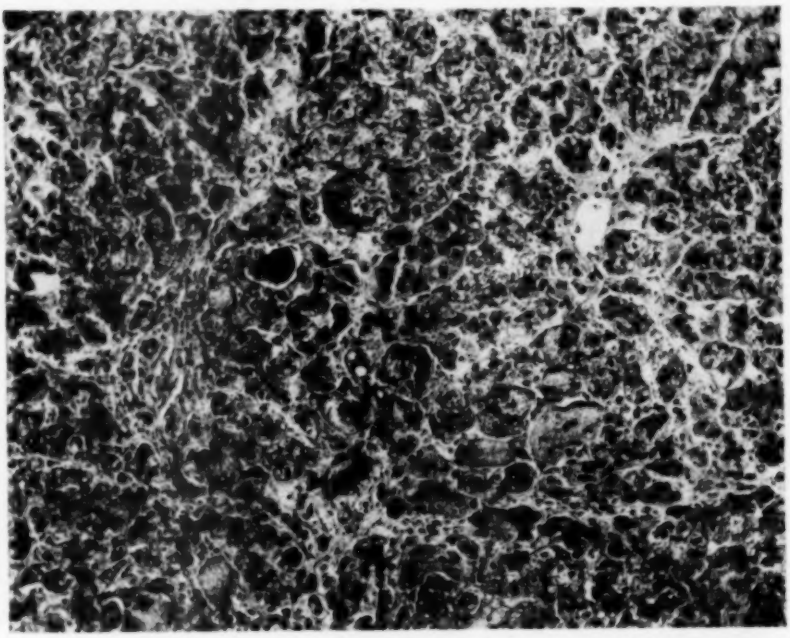

Fig. 1 (Case 3).—Specimen from liver obtained for biopsy from infant 5 months old. Marked swelling of parenchymal cells and distortion of liver cell cords are present. Dark eosinophilic, necrotic cells can be seen. Eosin-toluidine blue; × 150.

we believe fairly specific, histologic appearance could be separated from the material. We were first attracted to this group by the presence of large numbers of multinucleate giant parenchymal cells. On careful histologic study, we found that a large proportion of such livers also possessed other features which further delineated them, giving a highly specific pattern.

This group of liver specimens contained the following characteristics, with, of course, minor variations in individual cases. The general achitecture of the liver was preserved (Fig. 1), with maintenance of the relationships of the portal triads

4. Taft, E. B.: Stain Technol. **26**:205, 1951.

and central veins. No regenerative nodules or reconstruction of the portal areas was present. Frequently the lobules were somewhat shrunken because of diffuse loss of parenchymal cell elements. The portal triads were widened to a slight degree, but there was only a minimal degree of bile duct proliferation. No dilatation of the bile ducts was evident, and in only a single instance was there evidence of bile stasis in the ducts of the portal triads. In this instance, bile duct proliferation was more obvious, and bile stasis in the lobule was more frequently intracanalicular than intracellular. Infiltration of the portal areas was present in all cases, but only to a moderate degree. The infiltrate consisted of lymphocytes, eosinophilic leucocytes,

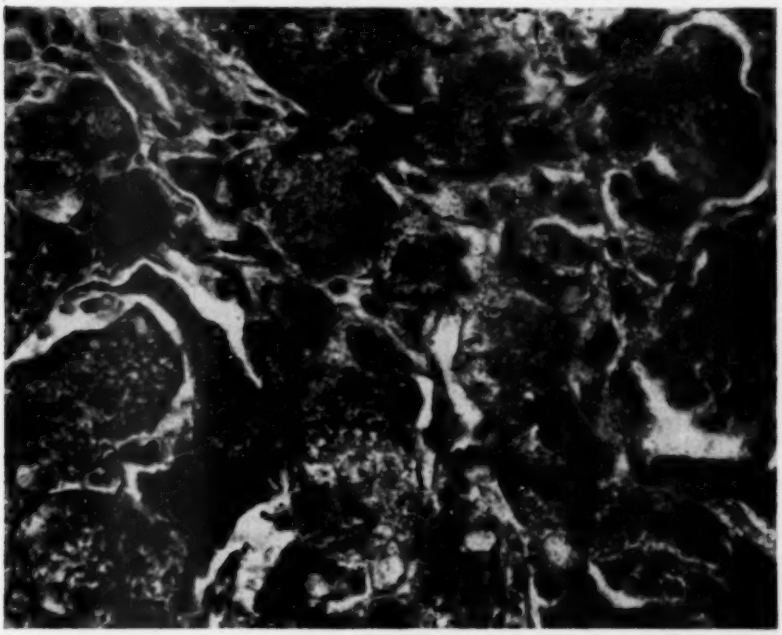

Fig. 2 (Case 3).—Specimen from liver obtained for biopsy. Perinuclear basophilia, as well as necrobiosis of individual cells (on right), is apparent. Eosin-toluidine blue; × 500.

and polymorphonuclear cells. In several instances, myelopoiesis was a feature of the portal triads. Although the grosser lobular architecture was preserved, the distortion of the liver cell cord pattern was extreme. This distortion was caused by the great variation in liver cell size. The multinucleate giant parenchymal cells were diffusely distributed throughout the lobules, and there was a slight tendency for increased numbers to be present in the central and peripheral areas. These cells, frequently as large as 400 μ in diameter, had as many as 20 small, often poorly outlined, nuclei. Frequently the nuclei were bunched in the middle or at one end of the cell. No evidence of mitotic or amitotic activity could be found in these cells. The cytoplasm was mostly clear, with large chunky basophilic masses or with a fairly sizable mass of perinuclear basophilic material. In most instances, the cells contained large

amounts of glycogen, but only occasional cells showed the presence of appreciable amounts of stainable lipid. Bile was present in small drops in many of these cells. Both the nuclei and the cytoplasm in scattered giant cells showed evidences of degenerative change. In the nuclei (Fig. 2), this change consisted of loss of the basophilic chromatin stain, disappearance of the nucleoli, and eventual breaking up of the nuclear membrane. Occasionally, this change affected a single nucleus in a group of several. In the cytoplasm, the earliest degenerative changes were those of focal acidophilia. This eosinophilic hyaline refractile change gradually spread to include the whole cell, with loss of the cytoplasmic boundary and, often, apparent hemorrhage into the cell or infiltration of it by polymorphonuclear cells. No large areas of coagulative necrosis were encountered, though in some instances there was

TABLE 1.—*Cases of Hepatitis in Infants Having Liver Biopsy*

Case	Age at Onset of Jaundice	Age When First Seen at Hospital	Acholic Stools	Erythroblastosis Fetalis	Hemoglobin Level at Entry, Gm./100 Cc.	Serum Alkaline Phosphatase Level, Bodansky Units	Serum Bilirubin, Mg./100 Cc., Direct/Indirect	Serum Cephalin-Cholesterol Flocculation Test	Comment
1	6 hr.	2 mo.	Yes	Yes	11.0	10.6	6.7/15.6	1+	Jaundice cleared 3 months after operation; well at 17 mo.
2	8 days	10 wk.	Yes	No	11.0	....	150 (icteric index)	...	No follow-up
3	10 days	4 mo.	Yes	No	9.0	5.8	5.8/11.1	±	Died at 5 mo. in liver failure
4	7 days	3 mo.	Yes	No	....	....	5.8/10.2	3+	No follow-up
5	6 wk.	5 mo.	Yes	No	11.0	23.1	9.5/18.7	Negative early; 2+ late	Jaundice cleared 6 wk. after operation; died with cirrhosis at 2 yr.
6	12 hr.	1 mo.	Yes	No	13.0	....	3.12/7.96	2+	Jaundice cleared 2 mo. after operation; well 3 yr. later
7	Birth	2 wk.	Yes	?	6.0	7.6	.....	...	Well 10 yr. after operation; evidence of hemolytic anemia
8	10 days	7 wk.	Yes	No	12.0	....	9.2/17.3	2+	No follow-up
9	3 days	2 mo.	Yes	No	10.0	....	4.3/7.2	...	Jaundice cleared at 10 mo.; died at 2¾ yr. with cirrhosis
10	5 wk.	2 mo.	Yes	No	10.0	....	.....	...	Jaundice cleared 1 wk. after operation; well at 1 yr.
11	Birth	2 mo.	Yes	No	12.2	....	4.6/9.8	...	No follow-up

a focus of inflammatory cells occupying an area in the liver which was devoid of parenchymal cells. The foci were not limited to any particular area in the lobule. Occasionally, these areas would show some condensation of the reticulum. Central vasculitis was present in some specimens. Degenerative changes were not limited to the giant cells but were seen in mononucleate parenchymal cells as well. In some instances, the giant parenchymal forms appeared to predominate in the sections; in others, the giant cells were sparsely distributed. In all instances, however, the variation in cell size and "ballooning" of the cells was prominent. The Kupffer cells were swollen and often were filled with bile. Frequently, this phagocytosis of bile pigment was more prominent in the center of the lobules. In addition to the inflammatory foci in and near necrobiotic liver cells, there was moderate, diffuse infiltration of the lobules with lymphocytes, eosinophiles, and large monocytes. A not uniform, but striking and frequent, observation was the appearance of intrasinusoidal collections of erythropoietic foci. These consisted largely of normoblasts but contained occasional erythroblasts. Bile stasis within the canaliculi of the liver cords was a comparatively common finding but was not present in all cases. The intracellular bile

pigment was more conspicuous and was almost universally present. Measurements of nuclear size and cell size in two of the cases showed the average giant cell cytoplasm to be four to five times the size of the nongiant forms. The average number of nuclei ranged between six and nine in the giant cell forms. The nuclei of the multinucleate cells had an average size only slightly larger than that of the nuclei in single nucleated cells of the same liver (1.06 and 1.08 times the mononucleate cell nuclear diameter in the two specimens measured). All these values were based on 25 measurements each. A distribution curve of the nuclear size in these two specimens did not indicate that polyploidy was a frequent characteristic of the nuclei of either the giant cells or the nongiant cell forms.

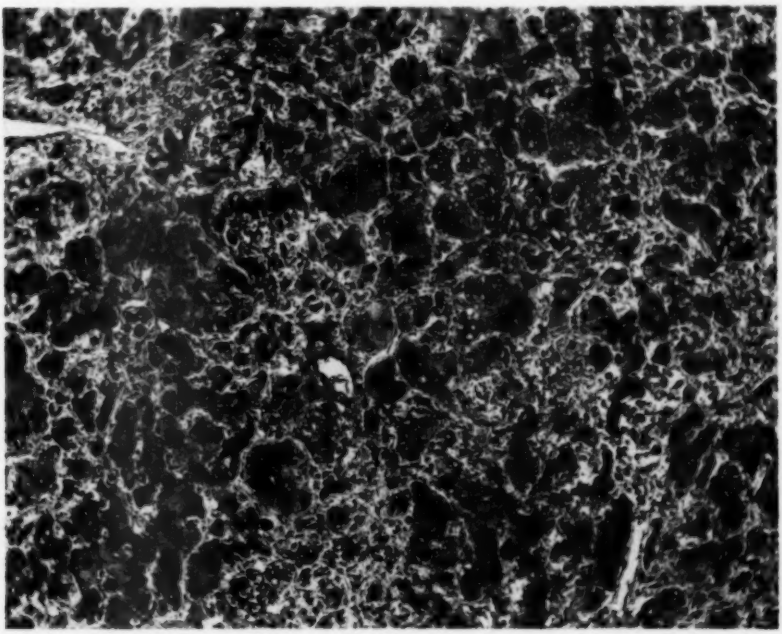

Fig. 3 (Case 3).—Specimen from liver obtained at postmortem examination from 6-month-old infant. Foci of complete loss of parenchyma are present, but the diffuse pattern and the predominance of "balloon" giant parenchymal cells remain. Hematoxylin and eosin; × 150.

In two cases which came to autopsy one month and two and one-half years, respectively, after the first biopsies we were able to observe the progression of the lesion and its termination in extensive cirrhosis. In the first instance (Case 3, Table 1), the infant died at 5 months of age in liver failure. The postmortem examination of liver sections showed a greater collapse of the reticulum and slightly larger foci of loss of parenchymal cells but the same general patterns described previously (Fig. 3). Multinucleate giant cell forms still were a dominant element in the histologic picture. In the second instance (Case 9) the liver was grossly distorted. There were large, irregular foci of regeneration (Fig. 4), and there were

moderate bile duct proliferation and marked variability in the size of the interlobular connective tissue bands. No trace of active hepatitis was present and no large multinucleate cells were observed, but large areas of liver parenchymal cell infarction were found; probably these were due to the combination of the hepatic vascular obstruction of the cirrhosis and the profound anemic shock the patient suffered terminally from bleeding esophageal varices.

Among the cases in which biopsy of the liver had been performed, 11 were found to conform to the histologic criteria described. When the records of these 11 cases were examined, a rather striking uniformity in their clinical picture was found. The following clinical history may be taken as a prototype of this group.

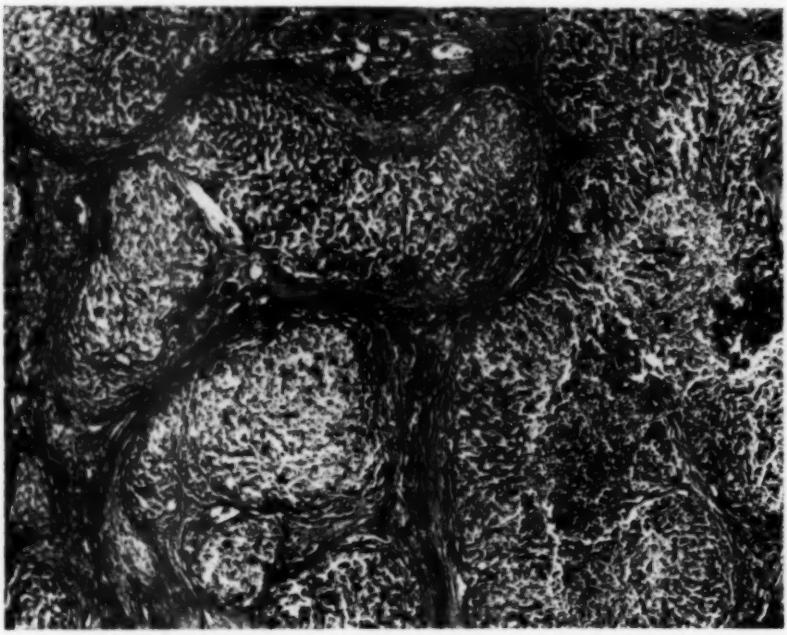

Fig. 4 (Case 9).—Specimen from liver obtained at postmortem examination from child 2 9/12 years old. Advanced cirrhosis is present. Extensive infarctive necrosis can be seen in the centers of the regenerative nodules. Little bile duct proliferation is seen. Hematoxylin and eosin; × 50.

REPORT OF A CASE

Case 3.—This infant was born by Caesarean section to healthy parents, though the mother's pregnancy was marked by vomiting and abdominal cramps during the first six months. A transfusion was given to the mother shortly before delivery. The infant appeared normal at birth. At the age of 10 days the infant's sclerae were icteric and the stools were clay-colored. On the fourteenth day, generalized icterus was present and the urine was dark. After this, the infant became progressively more jaundiced, though occasionally the stools were yellow. Low-grade fever was noted at this time, but the child was active and alert. The infant continued to be jaundiced until entry to the hospital, at 3 months of age. On admission to the hospital the child showed generalized lymphadenopathy, and the liver was 7.0 cm. below the costal

margin. The one-minute value for serum bilirubin was 5.8 mg. per 100 cc., and the total value was 11.1 mg. The thymol turbidity and thymol flocculation tests gave negative results; results of the cephalin-cholesterol flocculation test were equivocal, and the value for serum alkaline phosphatase was 5.8 Bodansky units. The urine did not contain urobilinogen but did contain bile. No Rh incompatibility was demonstrable. The Hinton test for syphilis gave negative results. Early in the infant's hospital course, he had a temperature as high as 103 F., some loose stools, and emesis. His temperature came down nearly to normal, and the diarrhea abated. The jaundice decreased slightly during the next month, and bile was demonstrated chemically in the stools, though they remained pale in color. During the sixth hospital week, laparotomy was performed. The liver was soft and symmetrical, but bile-stained; the gall bladder and bile ducts were normally formed, and cholangiography demonstrated a normal extrahepatic and intrahepatic biliary tree. After operation, the liver increased in size and the stools remained acholic. The infant lost weight, became listless and lethargic, and died in a comatose state 10 weeks after his entry to the hospital. At postmortem examination, no significant lesions were seen outside the liver, which was much enlarged and deeply bile-stained. The histologic observations have already been described.

SUMMARY OF CASE MATERIAL

Pertinent clinical data concerning the 11 cases in which liver biopsy had been performed revealed that in all infants the onset of jaundice occurred within the first six weeks of life, in nine within the first two weeks, and in four on the first day of life (Table 1). All had acholic stools at some time during their illness, and in nine the stools were free from bile from the onset of jaundice. Only in Case 3 was significant fever observed. The mothers' pregnancies were not marked by any unusual features, and none of the infants' siblings were known to be ill. In spite of their jaundice, these infants displayed good weight gains in 8 of the 11 cases. Indeed, the average delay of eight weeks from the onset of jaundice to the time of entry into the hospital attests to their relatively good health. At the time of entry, all the infants were jaundiced; in 10 of the 11 the liver was easily palpable, and in 3 the spleen was palpable also. Two infants displayed a slight bleeding tendency. The urine was examined for bile in eight instances, and bile was present in each case. Similarly, urobilinogen was absent from the urine in 8 cases. Laboratory examinations also revealed erythroblastosis of a mild degree, not requiring transfusion, in one infant and hemolytic anemia in another. In the latter, the peripheral blood contained 40 nucleated red cells per 100 white cells when the infant was 2 months old. The leucocyte counts usually were low, and the number of polymorphonuclear cells never rose above 60%. The serologic test for syphilis gave negative results in all cases. The serum alkaline phosphatase levels were elevated in one and normal in three. The values for serum bilirubin ranged from 3.12 to 9.5 mg. per 100 cc. for the one-minute test and from 7.2 to 18.7 mg. for the total. In all but two of the seven cases in which tests were carried out, the ratio of the direct to the indirect reading was more than 50%, and in these two cases it was more than 40%. The cephalin-cholesterol flocculation tests gave positive results before operation in four of the eight cases in which the test was made, and similar results were obtained with the thymol turbidity test. At operation, a patent biliary tree was demonstrated. The liver was described by the surgeon as being enlarged, smooth, soft, bile-stained, and without evident cirrhosis. Five of these patients are known to have lost their icterus at intervals of from 1 week to 10 months after leaving the hospital. In one additional case, the jaundice cleared subsequently;

in three cases no data are available. Four of the patients are known to be well from 1 to 10 years after discharge; three have died, one with continuing jaundice at 5 months of age (Case 3), and two with advanced cirrhosis at 2 years (Case 5), and 2¾ years (Case 9) of age, respectively. The postmortem findings in Cases 3 and 9 have already been mentioned. In four cases, no follow-up has been possible.

In the autopsy series, nine specimens (Table 2) were found to conform to the histologic criteria described (Fig. 5). The clinical records revealed that the onset of jaundice again ranged from the day of birth to the sixth week of life; in five of the nine it occurred within the first ten days. All but two patients had acholic stools during their illness, and in four, bile was absent from the onset of the jaundice. In four of the nine infants, the temperature was elevated to 100 F., and in one to 103 F.; this higher incidence of fever might have been expected in this

TABLE 2.—*Cases of Infantile Hepatitis Diagnosed at Postmortem Examination*

Case	Age at Onset of Jaundice	Age When First Seen at Hospital	Acholic Stools	Erythroblastosis Fetalis	Hemoglobin Level at Entry, Gm./100 Cc.	Serum Alkaline Phosphatase Level, Bodansky Units	Serum Bilirubin, Mg./100 Cc., Direct/Indirect	Serum Cephalin-Cholesterol Flocculation Test	Comment
12	6 wk.	8 wk.	Yes	No	....	....	24.9/29.7	...	Positive serologic test for syphilis; no postmortem evidence of syphilis pneumonia; recent acute infarction of intestine; death at 8½ wk.
13	2 mo.	2½ mo.	Yes	No	9	47	5.4/9.8	0.2+	Terminal diarrhea; death at 3½ months of age
14	3 wk.	5½ wk.	Yes	No	9	10.85	17/.. 100 icteric index	...	Terminal diarrhea with colonic ulceration; death at 9 wk.
15	6 wk.	3 mo.	No	No	8.2	....	.....	Negative	Death at 5½ mo.; evidence of hemolytic anemia; exploration for atresia
16	Birth	6 wk.	Yes	Yes	3.0	....	.....	...	Terminal diarrhea
17	Birth	3 days	Yes	Yes	10.6	....	.....	...	Diarrhea before death, at 5 wk.
18	9 days	7 wk.	No	No	11.4	25.5	3.2/6.4	Negative	Exploration; questionable mucous plug in stenotic duct; died 2 mo. after second operation
19	12 hr.	3 mo.	Yes	No	11.1	31.7	.....	...	Terminal diarrhea; death at 4½ mo.
20	2 days	5 wk.	Yes	No	13.5	....	.....	...	Adrenogenital syndrome; death at 5½ weeks of age

group with an earlier, universally fatal outcome. The maternal histories were significant in two instances. In the first, the mother acquired syphilis in the first trimester of pregnancy. This was adequately treated with oxophenarsine hydrochloride (mapharsen*) from the third to the fifth month and with bismuth during the sixth and seventh months. Although results of a serologic test in the infant were positive, no gross or histologic evidences of syphilis were present. In the second case, the infant was premature (Case 19). Weight gains were recorded in only two of the nine cases, again an indication of the severer illness in this series. All infants were jaundiced at the time of entry into the hospital, though in two cases there was considerable fluctuation in the degree of jaundice during the hospital stay. The livers were palpable on entry in six of nine cases, and the spleen was palpable in two. In two there was a bleeding tendency.

Laboratory examinations revealed the urine to contain bile in all cases; urobilinogen was absent at some time in three of the six cases in which the urine was tested. Erythroblastosis fetalis of the Rh-incompatibility type was demonstrated in two of the infants, and nucleated red cells were present in the peripheral blood smears of these two as well as in a third, nonerythroblastotic, infant, one and one-half, two and one-half, and one month after birth, respectively. The leucocyte counts ranged somewhat higher in this group, though the percentage of polymorphonuclear leucocytes rose above 40% in only a single case. The serum alkaline phosphatase levels were elevated in five of the six recorded values. The total

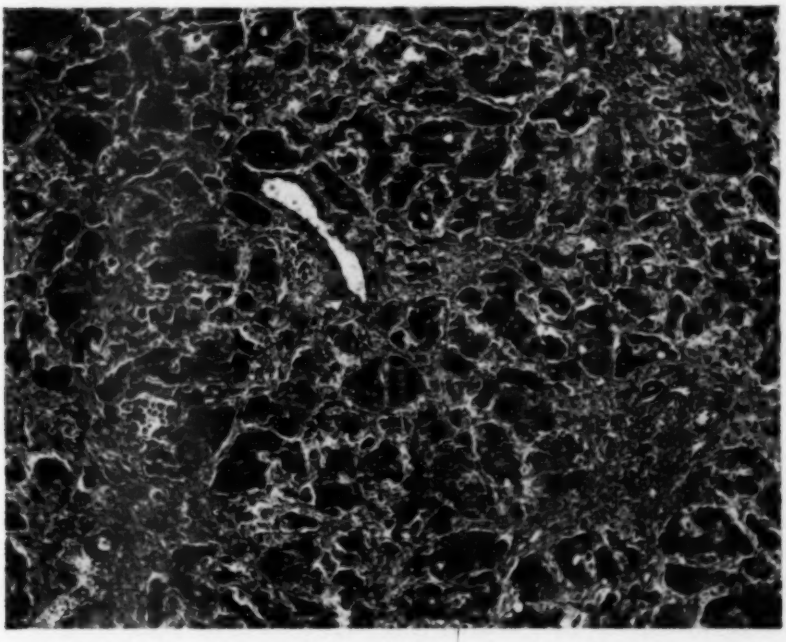

Fig. 5 (Case 18).—Specimen from liver obtained at postmortem examination from infant 2 months old. Giant parenchymal cells are numerous. Foci of liver cell necrosis are present but are small. A diffuse chronic inflammatory response is present. There is no proliferation of the bile ducts. Bile stasis can be seen in the canaliculi. Hematoxylin and eosin; × 150.

serum bilirubin values ranged from 6.4 to 29.7 mg. per 100 cc., and in all four instances in which the examination was made the direct value was more than 50% of the indirect. Results of the cephalin-cholesterol flocculation test were negative in all four recorded cases; the thymol turbidity test gave positive results in only one of the three cases in which it was performed. In five cases, diarrhea developed at some time after the onset of jaundice, and in four of the nine it was a prominent feature of the last few days of life. In two of these exploratory operation for suspected biliary atresia was carried out. In one, the bile ducts were believed to be normal; in the second, the common duct was believed to be stenotic in the region of the head of the pancreas, though it was not dilated proximally. The common

bile duct was divided and ligated, and a choledochoduodenostomy was performed. It is interesting to note that a considerable amount of bile-stained fluid was present in the peritoneal cavity at exploration.

These infants all died in the hospital during the course of their first and final illness. At postmortem examination, the principal findings were related to the liver. This was enlarged at least one and one-half times in five of the seven instances in which the organ was weighed. The livers, like those examined at operation, were smooth, bile-stained, and non-nodular. The bile ducts were demonstrably patent in all but the single case in which the common duct had been previously ligated. The other significant postmortem findings included bronchopneumonia and interstitial pneumonia, and, in addition, acute ulceration of the bowel, small venous infarction of the sigmoid colon of an acute nature, and hyperplastic adrenals of the adrenogenital syndrome were found in single cases. No evidence of overwhelming sepsis was found in any of the cases. In addition to the records of those cases which had the characteristic histologic features mentioned previously, the clinical records of cases with lesions approaching those, but not similar to them, were reviewed also. In all these cases, the histologic findings of biliary cirrhosis, the diffuse or focal nonspecific or periportal hepatitis of sepsis, biliary stasis, hepatoma, hemangioendothelioma, and so forth, fitted in well with the clinical findings. Two surgical biopsy specimens, with explored and patent extrahepatic duct systems, were disturbing until they were recognized as examples of intrahepatic biliary atresia.[2]

COMMENT

The histologic similarity of the sections in these cases to those described in biopsy specimens from patients having virus hepatitis of either epidemic or serum origin is striking.[3] Indeed, the histologic criteria of Weisbrod, Schiff, and their associates [4,5] can be checked off almost item by item. However, the liver lesions at postmortem examination in our material are at variance with the classic postmortem lesions so well described by Lucké [6] and Lucké and Mallory.[7] They differ in the lack of widespread central necrosis and complete destruction of the liver cells of the lobule. We believe that this is, in some manner, related to the blood supply and resistance of the livers of infants to autolysis, since symmetrical central necrosis is an unusual finding in infants and children less than 2 years of age. If widespread necrosis, from whatever cause, does occur in the livers of infants, it appears to be either focal in character or completely nonsymmetrical and multilobular in nature. Undoubtedly, much of the massive necrosis in adult hepatitis is terminal, since it is not seen in liver biopsy specimens and since massive coagulative necrosis is not regarded as a feature compatible with viral hepatitis.[8] None

5. (*a*) Roholm, K., and Iversen, P.: Acta path. et microbiol. scandinav. **16**:427, 1939. (*b*) Dible, J. H.; McMichael, J., and Sherlock, S. P. V.: Lancet **2**:402, 1943. (*c*) Axenfeld, H., and Brass, K.: Frankfurt. Ztschr. Path. **58**:220, 1944. (*d*) Mallory, T. B.: J. A. M. A. **134**:655, 1947. (*e*) Popper, H.: In Liver Injury, Transactions of the Seventh Conference, New York, Josiah Macy, Jr. Foundation, 1948, p. 66. (*f*) Popper, H.; Steigmann, F., and Szanto, P. B.: Am. J. Clin. Path. **19**:710, 1949. (*g*) Weisbrod, F. G.; Schiff, L.; Gall, E. A.; Cleveland, F. P., and Berman, J. R.: Gastroenterology **14**:56, 1950.

6. Lucké, B.: Am. J. Path. **20**:471, 1944.

7. Lucké, B., and Mallory, T.: Am. J. Path. **22**:867, 1946.

8. Popper, H., and Franklin, M.: Arch. Path. **46**:338, 1948.

of our patients had a fulminant course, which may give a picture of massive parenchymal cell destruction.[7] One other disturbing feature is the absence of regenerative lobules. In some of the cases of epidemic hepatitis reported by Lucké [6] and by Stokes and Miller,[9] regenerative lobules are reported as being present at the end of 11 to 22 days. Yet, in the present series, with cases of longer duration, no regenerative lobules were seen. Again, this lack probably is related to the absence of large, confluent areas of necrosis in our material.

A similar group of cases was reported by Stokes and his associates.[8] The virus of serum hepatitis was transmitted to volunteers from one of the infants in their series and from its mother. Stokes [4,10] also reported that people harboring the virus of serum hepatitis often have no history of jaundice. Therefore the absence of any history of jaundice in the mothers in this series does not rule out the possibility of a transplacental transmission of the virus of serum hepatitis to the infants under discussion.

In these patients the high percentage of negative results to tests for active hepatitis, such as the thymol turbidity and cephalin-cholesterol flocculation tests, is worthy of comment. Active and severe parenchymal cell damage, such as is present in these cases, is regarded as having a high degree of correlation with positive results for serum flocculation tests.[5e,f] In infants and young children, however, experience has shown that such tests are unreliable and often results are negative in cases of known hepatitis.[11] Ducci [12] suggested that the absence of bile from the intestine may have some causal relationship with the negative results for flocculation tests in hepatitis, and that after the period of acholic stools has passed the flocculation test may give positive results. It should be remembered that the stools from all but 2 of the 20 patients in our series were acholic.

It may be objected that these cases may represent examples of toxic or "parenteral hepatitis" due to overwhelming sepsis. Ladd [13] stated that acholic stools do not occur in this condition. In the infants under discussion, jaundice was the chief early symptom, acholic stools were present in all but 2 cases, and in no case were there postmortem evidences of overwhelming sepsis or severe infectious processes in other organs.

Perhaps we have overemphasized the diagnostic importance of the multinucleate giant cell in this group of cases. At the outset it may be said that similar multinucleate giant cells are present in cases of biliary atresia; among the sections from infants with biliary atresia which we examined, a few giant parenchymal cell forms were encountered in a third of the cases. These were congregated about the central veins and displayed fairly obvious degenerative changes without marked "ballooning." In only one case of biliary atresia were they a "preponderant" element, a feature suggestive to Stokes and Wolman and their associates [8] of a virus origin. The other histologic features of this single case, the lack of lobular inflammatory

9. Stokes, J. F., and Miller, A. A.: Quart. J. Med. **16**:211, 1947.

10. Stokes, J. F., Jr.: Studies on Virus Hepatitis, delivered as Alpha Omega Alpha lecture, Harvard Medical School, March 20, 1952.

11. Weller, S. D. V.: Great Ormond Street J. 1951, No. 1, p. 26.

12. Ducci, H., in Ciba Foundation Symposium: Liver Disease, Philadelphia, The Blakiston Company, 1951, p. 57.

13. Ladd, W. E.: Ann. Surg. **102**:742, 1935.

infiltration, the lack of "ballooning" of the cells, the marked proliferation of bile-duct and portal fibrous tissue, and the extensive bile stasis, all served to distinguish it from our group of selected cases.

The significance of multinucleate giant cells in the human liver is subject to debate. Such cells are not seen in experimental regenerating rat liver after partial surgical excision, and the number of binucleate cells may actually decrease during the most rapid phase of regeneration.[14] However, in human material they have been considered as evidence of regeneration.[15] We have seen neither mitotic figures nor evidence of amitotic division in the multinucleate giant cells in this material. The measurements of nuclear size which we carried out indicate that the nuclei of multinucleate cells are slightly larger than those in the mononucleate cells, though the differences are not of the order of magnitude usually associated with rapidly dividing cells. Weisbrod and his associates[5g] and Klemperer and his co-workers[16] both have considered the formation of multinucleate cells to be a degenerative phenomenon. Weisbrod and his associates[5g] believed that these cells formed as a result of a reaction to necrosis. Klemperer and his co-workers[16] noted, as we have, that these cells often show eosinophilic hyaline degenerative changes. He felt that they arose by amitotic nuclear division without accompanying cell division and were a frustrated attempt at regeneration. Mallory[5d] concurred in this interpretation. Since these cells so often assume the shape of the liver cell cords rather than the rounded shape of an independent cell, we wonder whether they may not arise sometimes by dissolution of cell boundaries without nuclear division. The multinucleate cells of liver cell carcinoma appear to arise out of multipolar spindles, often from a giant mononucleate cell,[17] neither of which cell types are present in this series of cases.

In the group of 20 cases which we have presented, there are three with the clinical and serologic criteria of erythroblastosis fetalis due to Rh incompatibility. Two of these have been reported previously by one of us,[18] in a series outlining the progression of changes in the liver to cirrhosis in a group of fatal cases of erythroblastosis. Many of the livers in the present series showed evidences of erythropoiesis in the sinusoids, and in four instances nucleated red cells were present in the peripheral blood in considerable numbers two weeks and longer after birth. Only one of these was known to be a case of erythroblastosis, though as a rule in cases of erythroblastosis the nucleated red cells have disappeared from the peripheral blood by this time.[19] This suggests an active hemolytic process and a retention jaundice element in some of these cases, in addition to the regurgitation jaundice.

The rapid progression to cirrhosis following hepatitis has been noted by Krarup and Roholm[20] and Wallgren.[21] In the present series, definite cirrhosis with portal

14. Beams, H. W., and King, R. L.: Anat. Rec. **83**:281, 1942.
15. Ashworth, C. T., and Reid, H. C.: Am. J. Path. **23**:269, 1947.
16. Klemperer, P.; Killian, J. A., and Heyd, C. G.: Arch. Path. **2**:631, 1926.
17. Rowen, H. S., and Mallory, F. B.: Am. J. Path. **1**:677, 1925.
18. Craig, J. M.: Arch. Path. **49**:665, 1950.
19. Diamond, L. K.: Personal communication to the authors.
20. Krarup, N. B., and Roholm, K.: Acta med. scandinav. **108**:306, 1941.
21. Wallgren, A.: Acta pædiat. **9**(Supp. 2):1, 1930.

obstruction was noted in two patients within five and seven months of operation respectively, at which time no cirrhosis had been present.

The role of inspissation of bile or mucus in the biliary passages as a cause of obstructive jaundice is a point which has been debated considerably. In cases of epidemic hepatitis, of course, its role has been denied repeatedly, although Mallory [5d] admits that inspissated bile may be encountered occasionally at the ampulla in fatal cases of epidemic hepatitis. In their book on "Abdominal Surgery of Infancy and Childhood," Ladd and Gross [22] cited a group of cases in which this diagnosis had been made. In only a single case in our present series, in which the common duct was said to be stenotic by the surgeon, was any suggestion made by him that a mucous plug might be present. In this case and in the other 19 cases, as well as in the cases of intrahepatic biliary atresia where severe jaundice and acholic stools were present with a patent extrahepatic duct system, the lesions in the liver seemed adequate to account for the clinical findings. The histologic finding of bile plugs in the canaliculi is a common one in erythroblastosis of the icterus gravis type, yet only a small number of these infants show serious derangements in their liver function over any prolonged period. Perhaps this histologic finding should be regarded as a result rather than a cause of the jaundice. Since biliary atresia may give rise to irreparable damage to the liver within two and one-half months of birth, early examination and, if necessary, exploration with liver biopsy appears to be indicated in any case of prolonged neonatal jaundice in which the diagnosis is not made clear by other means.

CONCLUSIONS

A group of 20 cases of hepatitis occurring early in infancy are reported. On admission to the hospital, all of the infants had severe jaundice and bile in the urine and all but two had acholic stools. Results of liver tests for hepatitis were negative in a high percentage of cases. In 13, in which there was a tentative diagnosis of biliary atresia, exploratory operations were performed, but in no case was the biliary tree atretic. Histologically active hepatitis having the characteristics of viral hepatitis was found in all 20 cases. Twelve patients died, 10 in their first illness and 2 after the development of cirrhosis and portal obstruction. Four are living and well, and four have not been traced. From these data, it appears that hepatitis having the histologic appearance of viral hepatitis is a not too infrequent finding in early life and must be considered in any differential diagnosis of icterus in the neonatal period.

22. Ladd, W. E., and Gross, R. E.: Abdominal Surgery of Infancy and Childhood, Philadelphia, W. B. Saunders Company, 1941, p. 274.

EFFECTS OF CORTISONE ON NONPERFORATING WOUNDS OF CORNEA IN NORMAL AND SCORBUTIC GUINEA PIGS

AELETA BARBER, Ph.D.
AND
W. G. NOTHACKER, M.D.
NEW ORLEANS

CONSIDERABLE information has accumulated in the literature indicating that cortisone retards wound healing by interfering with fibroblastic proliferation. According to Spain [1] and his associates this influence is manifested only during the early stages, no fibrolytic action being involved. The alteration in the response of collagen has been considered similar to the recognized effects of vitamin C deficiency, both resulting in delayed wound healing by the elements of connective tissue.[2] Nearly all the experiments reported have dealt with body wounds in humans or in small animals and have been complicated by the presence of vascular tissue.

The experiments in this investigation were designed to determine whether or not the administration of cortisone would alter wound healing in an avascular tissue such as the cornea of normal and scorbutic guinea pigs.

MATERIALS AND METHODS

Male guinea pigs, weighing between 150 and 250 gm., were fed ad libitum the scurvy-producing diet described by Wolbach and Howe,[3] and control animals of the same average weight were maintained on a standard fortified diet. Vitamin C deficiency was estimated by loss of weight, bloody stools, visceral and joint hemorrhages, and predictable death on an approximate date of the scorbutigenic regimen.

A nonperforating wound, in the form of a sagittal incision approximately one-third the depth of the stroma, was made in the center of the cornea of only one eye of 12 untreated control animals and in both eyes of 78 treated animals. Wounding of the cornea was done on the 5th, 7th, and 10th days of the scorbutigenic diet and 24 hours after initiation of cortisone therapy. A 4% solution of cocaine hydrochloride was used for local anesthesia, and the eyes were washed before and after the operation with a 1:4,000 solution of benzalkonium (zephiran®) chloride twice daily. No instance of infection occurred in any of these animals.

Presented before the American Society for Experimental Pathology April 16, 1952, New York.

From the Department of Pathology, Louisiana State University School of Medicine and the Charity Hospital of Louisiana.

1. Spain, D. M.; Mollomut, N., and Haber, A.: Biological Studies on Cortisone in Mice, Science **112**:335-337, 1950.

2. Ragan, C.; Howes, E. L.; Poltz, C. M.; Meyer, K., and Blunt, J. W.: Effect of Cortisone on Production of Granulation Tissue in the Rabbit, Proc. Soc. Exper. Biol. & Med. **72**:718-721, 1949. Hyman, G. A.; Ragan, C., and Turner, J. C.: Effect of Cortisone and Adrenocorticotrophic Hormone (ACTH) on Experimental Scurvy in the Guinea Pig, ibid. **75**:470-475, 1950. Schaffenburg, C.; Masson, G. M. C., and Corcoran, A. C.: Interrelationships of Desoxycorticosterone, Cortisone and Vitamin C in the Genesis of Mesenchymal Lesions, ibid. **74**:358-362, 1950.

3. Wolbach, S. B., and Howe, P. R.: Intercellular Substance in Experimental Scorbutus, Arch. Path. **1**:1-24, 1926.

Cortisone acetate in isotonic sodium chloride solution was used for topical administration and a suspension of cortisone acetate in isotonic sodium chloride solution was given by subcutaneous injections in the amounts of 0.1 mg., 1 mg., and 10 mg. daily. Cortisone, both topically and systemically, was given to an equal number of animals maintained on the standard fortified diet and to those fed the scorbutigenic diet.

The animals were killed at intervals of 8, 12, and 24 hours for study of the early stages of healing and 2, 4, 6, 8, 10, 12, 14, 18, and 22 days for the later stages. Ten per cent formalin was used as a fixing agent, and paraffin sections were cut at 4 μ. The histologic techniques used included hematoxylin-and-eosin staining, use of Mallory's connective-tissue stain, and the combined silver and connective-tissue method of Masson.

RESULTS

Macroscopic Observations.—The four plotted lines in Figure 1 represent the average body weight of four groups of guinea pigs. Two of these groups were maintained on a normal laboratory chow, and the other two groups were fed the

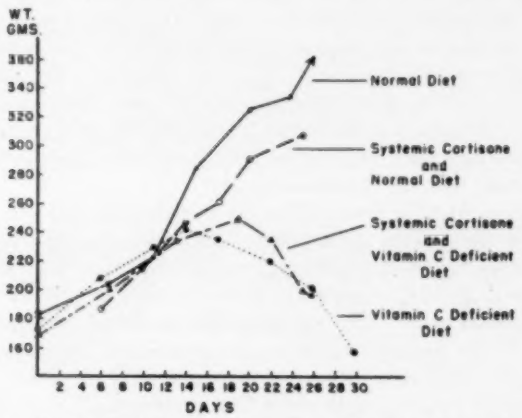

Fig. 1.—Growth of guinea pigs on various diets and treatments, as shown by average of body weights in experimental groups.

scorbutigenic diet. Cortisone in 10-mg. injections (0.1-mg. and 1-mg. injections had no effect) daily was given to one group on each type of diet; that is, cortisone was given to one group of animals on normal diet and one group on the vitamin-C-deficient diet, the other two groups serving as controls.

The body weight of guinea pigs fed the vitamin-C-deficient diet began to decrease sharply about the 14th day of the diet, and the decrease continued uninterruptedly until death on approximately the 25th to 30th day. Cortisone in doses of 10 mg. daily failed to effect the normal increment in body weight of the animals on normal diet or to prevent the expression of symptoms of scorbutus in the animals fed the vitamin-C-deficient diet. The sharp drop in body weight of the scorbutic animals was delayed for four to five days in the latter group.

Grossly the scars in the corneas of animals on normal diet treated with cortisone appeared edematous or raised, while those of animals on vitamin-C-deficient diet with and without cortisone appeared very white and flat.

Microscopic Observations.—Regeneration of the corneal epithelium was similar in all the groups. In most instances the surface of the wound was partially covered 12 hours after operation and was completely covered at the end of 24 hours. Neither cortisone nor vitamin C deficiency appeared to alter the growth or morphology of the epithelium.

Control Animals.—A marked generalized infiltration of eosinophilic cells was present throughout the corneal stroma of the controls eight hours after operation. The number of these cells gradually decreased, and only a few were found at the end of 48 hours.

A definite glass membrane comparable to Bowman's membrane in the human eye could not be identified in the guinea pig's cornea, but in sections stained by the silver and combined silver and connective tissue methods, a layer of silver-positive material was located just deep to the corneal epithelium. In wounds of eight hours or older, silver-positive material was present at the cut edges of the corneal lamellae at their points of contact with the overlying corneal epithelium where it had grown into the defect. This material was in direct continuity with the normally occurring silver-positive layer at the outer margin of the corneal stroma (Fig. 2 *A*).

At the end of the second day fibroblasts had accumulated in the lamellar spaces around the periphery of the wound and an occasional binucleated form was noted. The silver-positive material was more abundant and formed irregular masses from which a few small fibers penetrated between the ends of the corneal lamellae where these were in contact with the epithelium.

Reticulum fibers could be identified in the wounds four days after operation, but there was no new collagen at this time. On the sixth day the reticulum fibers were more extensive and interlaced throughout the wound tract and a few very fine wavy collagen fibrils could be identified in the intercellular matrix. Also, the fibroblastic cells were more evenly distributed and were found in the wound tract.

By the 10th day the reticulum was reduced in amount and the collagen was more mature and was becoming arranged parallel to the surface of the cornea (Fig. 2 *B*). No noticeable change occurred in the wounds at the 18th and 22d days except that the collagen fibers in the wound tract approached more closely in their morphology and staining reactions those in the portions of the cornea away from the wound. A small amount of reticulum was still present at 22 days and the silver-positive layer beneath the corneal epithelium was almost reconstituted. No ingrowth of capillaries was noted at any stage of healing.

Cortisone with Normal Diet.—The infiltration of eosinophilic cells which occurs normally during the first few hours after operation was markedly reduced. Fibroblastic proliferation, based upon the relative number of mitotic figures and cellularity in older wounds, was also less than that of the controls receiving no cortisone (Fig. 3). Reticulum was present in the wounds on the fourth day but formed larger and more irregular masses, and the ground substance was more abundant but was free of fibrils. Collagen fibrils could not be identified until the 10th day, whereas they were found in the controls on the 6th day. The reticulum fibers and silver-positive material were consistently more granular and coarser on the 18th and 22d days than in control wounds. The collagen fibers were thin and the blue-staining matrix was scanty in 18-day wounds (Fig. 4 *A*).

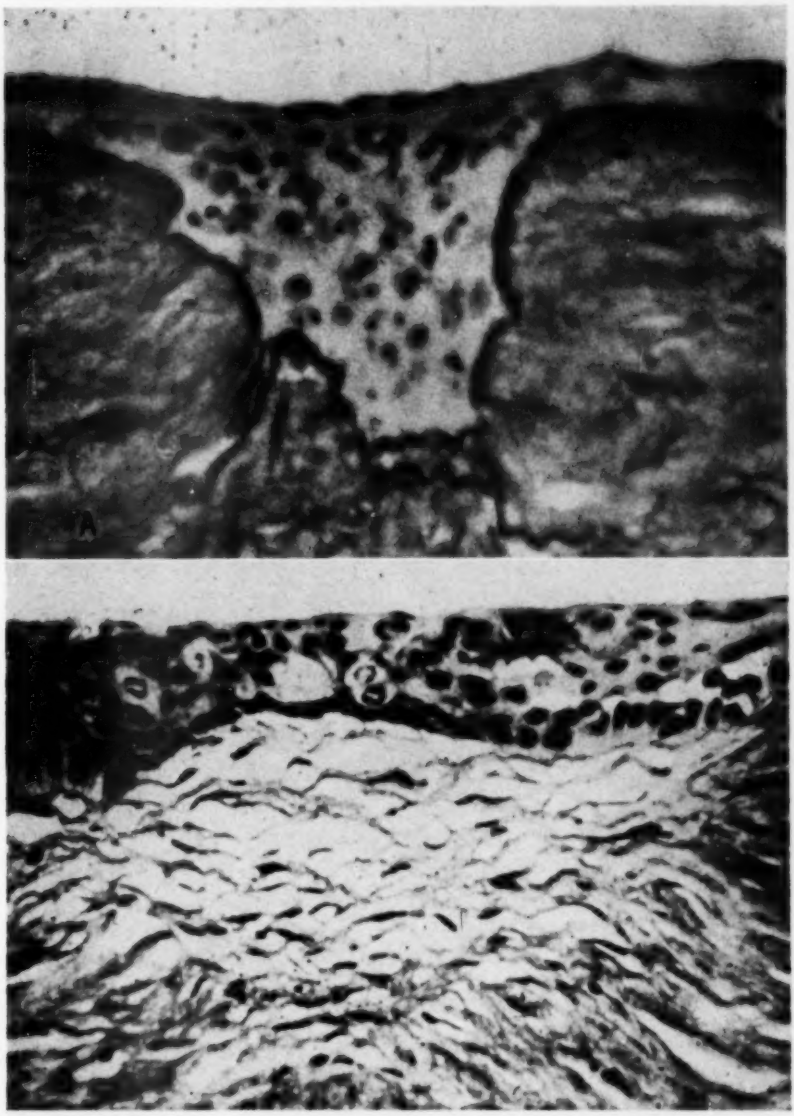

Fig. 2.—*A*, 24-hour wound in control guinea pig. The wound is completely filled with regenerating epithelium. A silver-positive layer is present between the epithelium and the cut ends of the corneal lamellae. Combined reticulum and connective-tissue stain; × 358. *B*, 10-day wound in control animal. Mature collagen has appeared and is arranging itself parallel with the surface of the cornea. Mallory's connective-tissue stain; × 158.

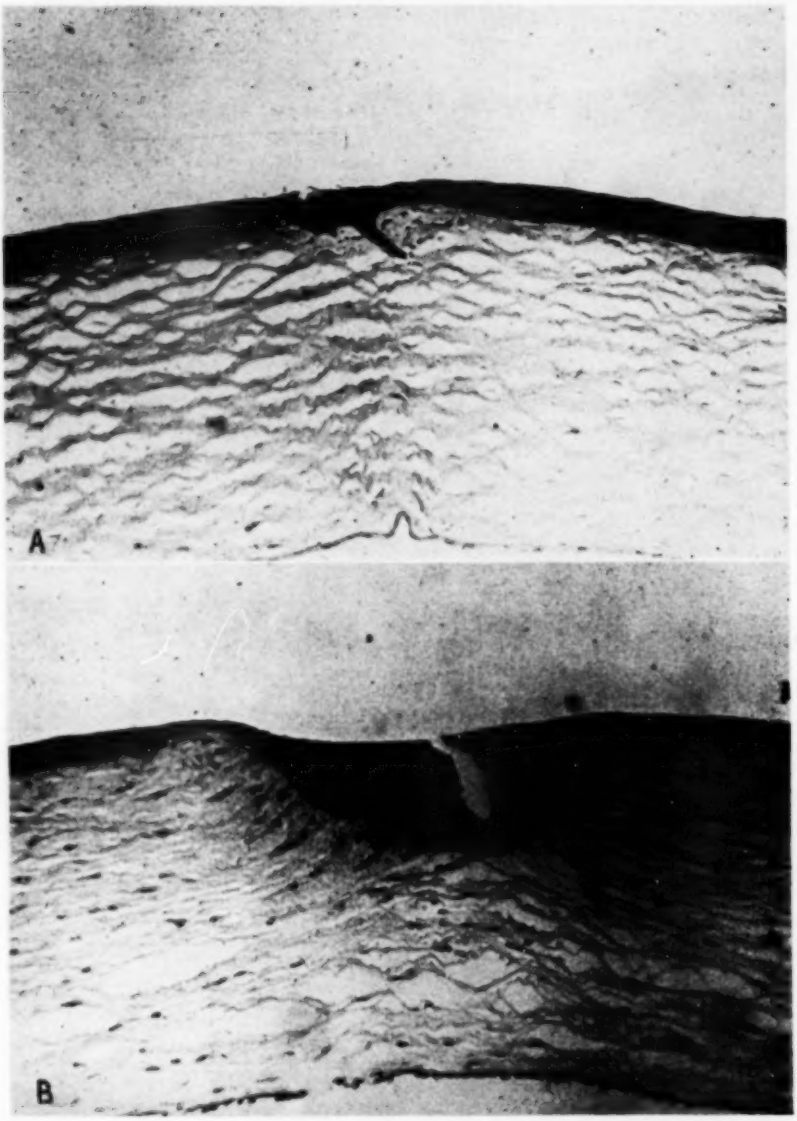

Fig. 3.—*A*, two-day wound in cortisone-treated animal on normal diet. Fibroblastic proliferation is retarded. Hematoxylin and eosin stain; × 80. *B*, two-day wound in untreated control. Fibroblasts are aggregated in the lamellar spaces at the margin of the wound, and an occasional binucleate form is seen. Hematoxylin and eosin stain; × 80.

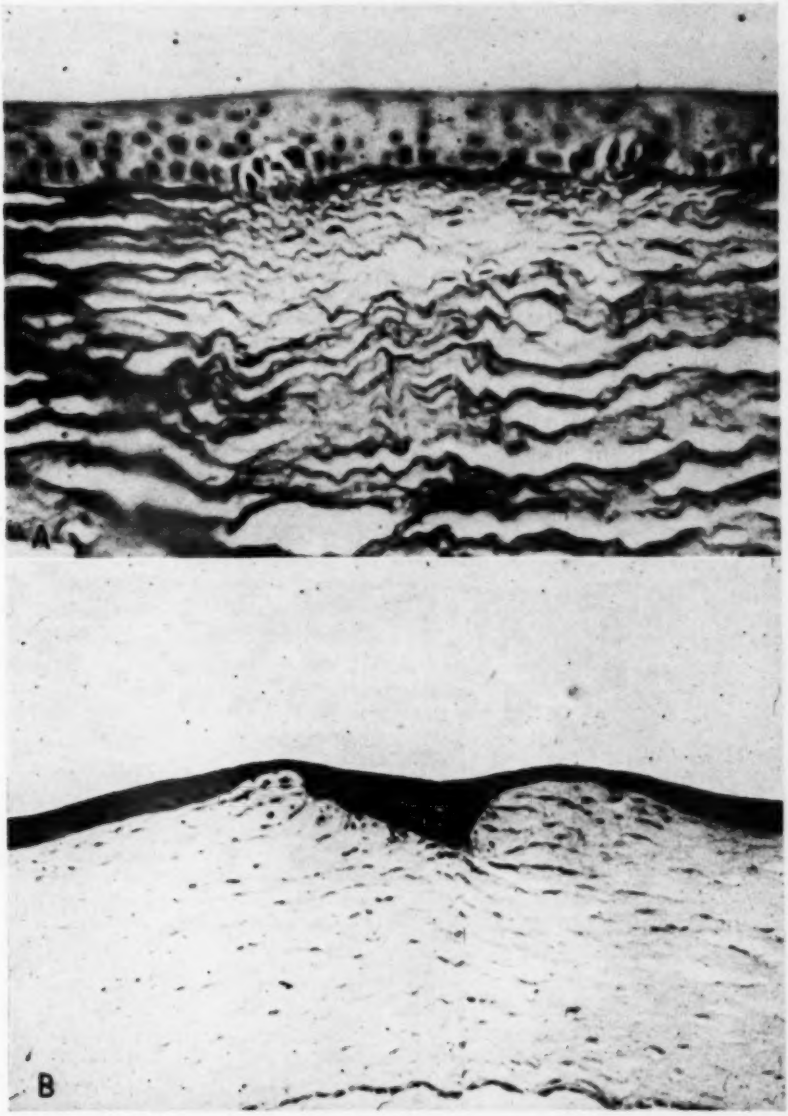

Fig. 4.—*A*, 18-day wound in cortisone-treated animal on normal diet. The ground substance is scanty and the collagen fibers are thin. Mallory's connective-tissue stain; × 158. *B*, two-day wound in cornea of vitamin-C-deficient guinea pig. Fibroblastic proliferation approaches that found in the untreated control. Hematoxylin and eosin stain; × 80.

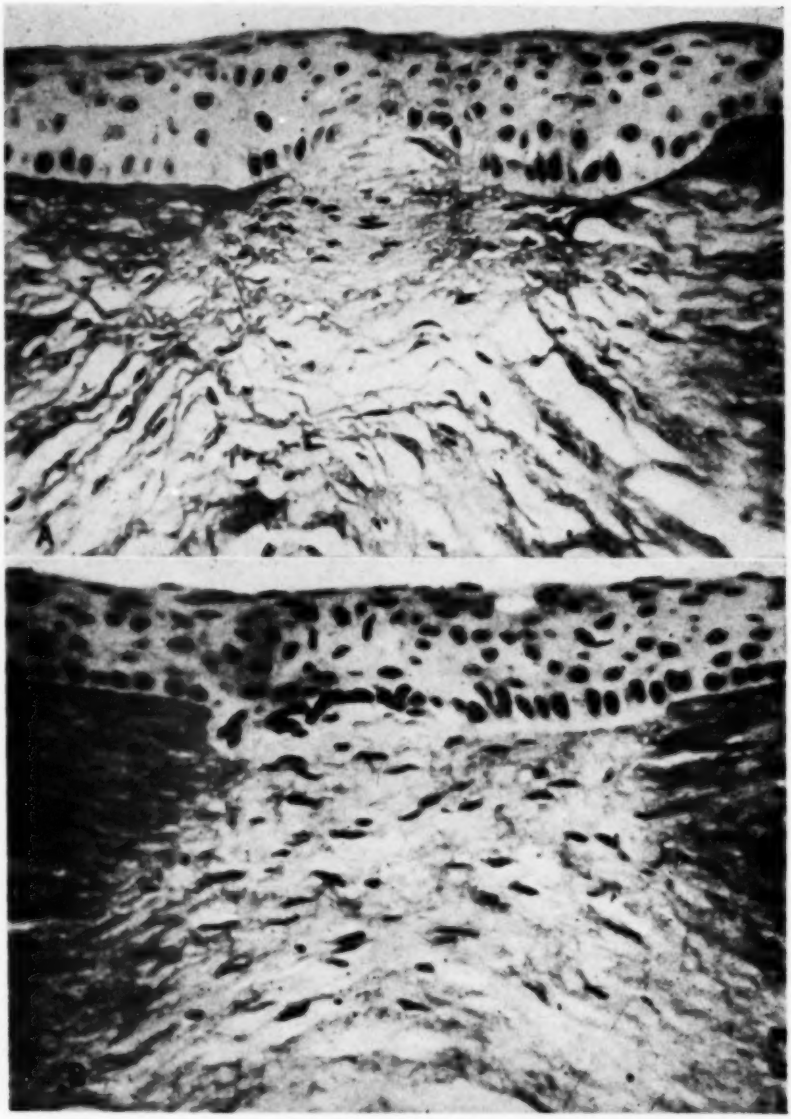

Fig. 5.—*A*, 18-day wound in vitamin-C-deficient guinea pig. The ground substance is scanty and the collagen fibers are immature and poorly oriented in relation to the surface of the cornea. Mallory's connective-tissue stain; × 158. *B*, 18-day wound in control. The wound is compact and the corneal lamellae are well oriented in regard to the surface of the cornea. Mallory's connective-tissue stain; × 158.

Vitamin-C-Deficient Diet Without Cortisone.—There was some reduction of eosinophilic cells during the first 12 hours but not as great as in the cortisone-treated animals. The cellularity of wounds at all stages was increased above that of the controls (Fig. 4 *B*). Reticulum could be identified on the fourth day, but the fibers were fewer and slenderer than in the controls. The ground substance was reduced but contained an occasional fibril. On the 18th and 22d day the cells and collagen fibers were not as well oriented as in the controls and the reticulum fibers were larger and coarser (Fig. 5).

Cortisone and Vitamin-C-Deficient Diet.—Cortisone did not alter the usual course of wound healing in scorbutic guinea pigs.

COMMENT

One of the problems growing out of the use of cortisone has been whether or not its effects are detrimental to wound healing in structures composed of connective tissue. Since the substantia propria of the cornea is composed almost entirely of collagenous tissue and is normally devoid of blood vessels, the healing process of noninfected wounds of the cornea should elucidate some of the problems connected with repair by connective tissue.

The clinical observations indicate that cortisone in the amount of 10-mg. doses daily was ineffective in preventing the symptoms of scorbutus (Fig. 1). The marked loss in body weight characteristic of guinea pigs fed the scorbutigenic diet was delayed for a few days, but no protective influence or "antiscorbutic" action could be demonstrated for cortisone in these experiments.

The similarity between the effects of cortisone and vitamin C deficiency on wound healing was not confirmed by our results. The cellularity of the wounds at all stages in vitamin-C-depleted animals was increased above that of the controls, while the cellularity of the wounds in cortisone-treated animals was definitely decreased. The increase in cellularity of the wounds in scorbutus is consistent with reports of other investigators on the role of vitamin C in wound healing.[4]

The results of our experiments on the effects of cortisone on wound healing agree with the results of previous studies in regard to the delay in the formation of the elements of connective tissue.[5] Histologic examinations were made in our series as long as 22 days after operation and showed that the wound was not completely healed, since argyrophilic precollagen fibers were still present in the controls and to a greater degree in the treated animals. Thus the delay in wound healing consisted in belated appearance of fibroblasts and in prolonged immaturity of collagen.

During the course of our experiments one additional finding developed in regard to the layer of silver-positive material which is located just beneath the corneal epithelium. The corneal epithelium grows very rapidly and covers a wound such as we have described in less than 24 hours. A group of untreated guinea pigs were wounded and killed at intervals of 30 seconds, 2 hours, and 4 hours. In animals killed immediately after wounding, the silver layer was seen to be interrupted at the site of the incision and the cut ends of the corneal lamellae did not react to silver

4. Hunt, A. H.: The Role of Vitamin C in Wound Healing, Brit. J. Surg. **28**:436-461, 1941.

5. Wolbach and Howe.[3] Wolbach, S. B.: Pathological Changes Resulting from Vitamin Deficiency, J. A. M. A. **108**:7-13, 1937.

salts. Two hours after wounding, the epithelial cells had extended over the margin of the wound and where they were in contact with collagen the points of contact were silver-positive. The development of the affinity for silver salts appeared to follow the advance of the epithelium, and when the wound was completely covered by epithelium the silver-positive layer was reconstituted in continuity with that peripheral to the wound (Fig. 2*A*). The contact between epithelium and collagen appears to produce or yield a reducing substance having an affinity for silver salts. Thus far we have not been able to locate in the literature a previous description of this finding.

SUMMARY

The effects of systemic and topical administration of cortisone on the healing of nonperforating wounds in the cornea of control and scorbutic guinea pigs have been studied.

The symptoms of scorbutus in guinea pigs were slightly delayed but not materially altered by daily injections of cortisone in doses of 10 mg.

The transient infiltration of eosinophiles in the cornea which occurs normally during the first 24 hours after operation on the guinea pig was reduced by both cortisone treatment and the vitamin-C-deficient diet, but was more marked in the animals receiving cortisone.

In guinea pigs on control diet, daily injections of 10 mg. of cortisone resulted in delayed wound healing occasioned by the belated appearance of fibroblasts and by the prolonged immaturity of collagen.

Similar treatment with cortisone of animals on scorbutigenic diet failed to change the effects of vitamin C deficiency, in which case there was a definite increase in fibroblastic proliferation.

One unanticipated finding developed: Whenever and wherever contact between regenerating epithelium and collagen occurred, the points of contacts were silver-positive. The suggestion is offered that this contact between epithelium and collagen produces or yields a reducing substance having an affinity for silver salts.

CHANGING VALUES OF NUCLEIC ACID OF CELLS IN BONE MARROW DURING DEVELOPMENT OF RED BLOOD CELL

MAUD L. MENTEN, M.D. Ph.D.
AND
MARY WILLMS, M.A.
VANCOUVER, B. C., CANADA

THE RED blood cell of man does not arise, like the Phoenix, full grown and *de novo* on the demise of its forbear, but undergoes a long progressive transformation before it reaches its terminal functional state. The sequence of morphologic changes which this cell manifests during maturation extends from its origin in the reticulum cells of the bone marrow through the free-moving basophilic hemocytoblast and progressively maturing types of normoblasts to the final erythrocyte devoid of nucleus. These changes, readily demonstrable by staining, reveal a morphologic gradient which is suggestive of a correlated chemical gradient. Such a chemical gradient may be divided into two parts: the first, growth with cellular division and increase in nucleic acid content of which the ribonucleic acid (RNA) is prominent, and the second, differentiation leading to formation of hemoglobin within the cell. Changes in the second part may be envisioned in sequence by loss of cytoplasmic ribonucleic acid with its basophilic conferring quality, gradual disappearance of mitochondria, depolymerization of nucleoprotein with increasing density of chromatin, and pyknosis. These changes ultimately lead to expulsion of the nucleus. This progression of nuclear changes does not seem compatible with the maintenance of a constant value of desoxyribonucleic acid reported for nuclei of mature diploid cells of different species by Boivin, Vendrely, and Vendrely,[1] Vendrely and Vendrely,[2] and Mirsky and Ris.[3]

The bone marrow is the site of origin not only of the red blood cells, but also of the granulocytes. If one assumes that similar growth principles underlie the development of the two series, then lack of any activating principle will arrest maturation, though not necessarily equally, in the two. The involvement of both erythroblasts and myeloblasts in pernicious anémia has been shown.

From the British Columbia Medical Research Institute and the Vancouver General Hospital.
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1. Boivin, A.; Vendrely, R., and Vendrely, C.: L'acide désoxyribonucléique du noyau cellulaire, depositaire des caracteres hérèditaires: Arguments d'ordre analytique, Compt. rend. Acad. sc. **226**:1061-1063, 1948.

2. Vendrely, R., and Vendrely, C.: La teneur du noyau cellulaire en acide désoxyribonucléique à travers les organes, les individus et les espèces animales, Experientia **4**:434-436, 1948; **5**:327-329, 1949.

3. Mirsky, A. E., and Ris, H.: Variable and Constant Components of Chromosomes, Nature, London **163**:666-667, 1949.

Among chemical estimations on bone marrow reported to date, none contain detailed figures for the nucleic acid content of successive types of developing cells. Published reports deal rather with mean cellular values. Davidson, Leslie, and White,[4] analyzing samples of bone marrow aspirated from normal persons as well as from patients suffering from various blood dyscrasias, found a wide range of values for desoxyribonucleic acid phosphorus and ribonucleic acid phosphorus, the average of which fell along a frequency curve with a contour similar in general conformation for both normal and pathologic conditions. These workers found in pernicious anemia an average value of total nucleic acid phosphorus of 2.595×10^{-9} mg.[5] per cell. Lutwak-Mann,[6] in studies on phosphorus metabolism of bone marrow from normal rats and rabbits, reported average values of desoxyribonucleic acid phosphorus, which became lower after irradiation and other drastic treatments. In normal rabbits and rats this worker gave the desoxyribonucleic acid phosphorus/ribonucleic acid phosphorus ratio as approximately 2.

In this paper are outlined the results of chemical analyses on bone marrow undertaken to determine whether the erythrocyte (with associated granulocyte) during the varying stages of progressive development maintains a constant amount of nucleic acid phosphorus or shows a succession of values. To obtain relatively high numbers of developing erythroblasts in progressive stages of maturation, samples of bone marrow were aspirated from patients with various blood dyscrasias, mostly macrocytic and hemolytic anemias, in which cells at certain stages of development accumulate. In the macrocytic dysplasias the early immature erythroblasts predominated. In the hemolytic anemias normoblasts were preponderant.

An advantage in the use of bone marrow for chemical analysis of cellular nucleic acid is the accuracy and ease with which the free separate cells of this tissue, compared with the cells of a homogenate derived from solid organs, can be enumerated.

Pernicious anemia may be looked upon as an arrest in the development of the erythrocyte due to a decrease of an enzyme (or co-enzymes) requisite for maturation. This arrest is correctable and terminable by therapeutically replenishing the lacking enzyme. Microchemical determinations of nucleic acids on cells of bone marrow from an untreated patient with this type of macrocytic anemia should reflect values for the red cell at this arrested level of development. Similar reasoning applies to other types of macrocytic or microcytic anemia. The series of values of nucleic acid which we have obtained and which is reported in this paper, we believe, represents the nucleic acid development of the nucleated progenitors of the red blood cells.

MATERIALS AND METHODS

The anemias, the majority of which were macrocytic in type, included in this report comprise those seen in the Vancouver General Hospital in patients presenting hematological dyscrasias and referred to the hematological department for detailed study. Most of the patients were

4. Davidson, J. N.; Leslie, I., and White, J. C.: The Nucleic Acid Content of the Cell, Lancet **1**:1287-1299, 1951.

5. We have expressed in milligrams the values reported in micrograms by Davidson and co-workers.

6. Lutwak-Mann, C.: Some Aspects of Phosphorus Metabolism in Bone Marrow, Biochem. J. **49**:300-310, 1951.

adults. When a diagnosis of the type of anemia was made by Dr. W. H. Perry, head of the hematological laboratories, and a recommended aspiration of bone marrow performed, extensive morphological examination and chemical analyses were carried out on the sample removed. As soon as bone marrow was aspirated, usually from the sternum, 1 ml. was carefully diluted 1:10 with water and another small sample, 0.1 to 0.2 cc., was shaken in a small test tube containing ammonium and potassium oxalate. In later experiments, the tubes were coated with silicone [7] previous to the addition of anticoagulant to mitigate rapid clotting, to which aspirated bone marrow is prone. The diluted aqueous sample was kept on ice until taken to the laboratory and, if analyses were not immediately started, was frozen and stored in the freezing compartment of a refrigerator or in the coldroom. On the smaller oxalated sample, dilution and enumeration of the nucleated cells were carried out in a Neubauer hemocytometer as for white cells. The number of cells per cubic millimeter was used for calculation of nucleic acid per cell. Differential cell counts were made on stained films of the bone marrow, which were lent by the hematological department. For chemical analyses of bone marrow the method of Schmidt and Thannhauser [8] with minor modifications was carried out on 2 cc. of the aqueous sample, as follows, and the results expressed in terms of nucleic acid phosphorus, as follows:

1. The acid-soluble constituents were removed first with 12% and then twice with 6% perchloric acid and centrifugation. The extracts were combined and formed the acid-soluble fraction, on which after acid digestion the phosphorus content was determined by the method of Fiske and Subbarow [9] on a Beckman spectrophotometer.

2. The phospholipids were removed from the residue with alcohol and hot ether-alcohol extraction. The various extracts were combined and digested with acid, and the phosphorus content was determined.

3. The residue from 2 contains the nucleic acids and phosphoprotein. The amount of phosphoprotein is the "total" phosphorus less the "nucleic acid" phosphorus of this residue. For estimation of total phosphorus a duplicate residue was obtained on a second 2 cc. aliquot of the original aqueous sample of diluted bone marrow. The duplicate residue was digested with 5 cc. of 5N H_2SO_4 and the phosphorus content determined. The phosphoprotein was invariably so small in amount that after a number of estimations analysis of this component was omitted. For the estimation of nucleic acids, the original residue remaining from 2 (and after the discontinuance of the phosphoprotein analysis, the duplicate residue as well) was incubated with 1 cc. of normal KOH at 37 C. for 20 hours for every 0.1 cc. of original bone marrow. At the end of this time, the cells were completely dissolved. This solution was acidified with 6N HCl to pH2 with thymol blue as an indicator. To the acidified solution was added one-tenth the volume of 25% trichloroacetic acid, with ice cooling and rapid stirring. The precipitate containing the desoxyribonucleic acid was removed with centrifugation and subjected to acid digestion and the phosphorus content determined. The remaining supernatant, containing the ribonucleic acid, was digested with acid and the phosphorus content determined. The duplicate analyses gave identical or only slightly differing values. In many cases the total phosphorus content was determined on an aliquot of the original aqueous sample as a check. In the separation of the two nucleic acids, care was taken to obtain complete precipitation of desoxyribonucleic acid with acid, and sufficiently prolonged centrifugation to bring down the entire precipitate.

A number of micro-Kjeldahl estimations for nitrogen were made on an aliquot of the aqueous sample of bone marrow, but because of the difficulty in completely eliminating the contaminating erythrocytes and plasma these analyses were discontinued.

Counts of reticulocytes and red and white cells in the blood and estimations of hemoglobin, as recorded on the patients' charts and in the files of the hematological department, have been included for comparison and possible correlation with the chemical values obtained. Differential

7. Jaques, L. B.; Fidlar, E.; Felsted, E. T., and Macdonald, A. G.: Silicones and Blood Coagulation, Canad. M. A. J. **55**:26-31, 1946.

8. Schmidt, G., and Thannhauser, S. J.: A Method for the Determination of Desoxyribonucleic Acid, Ribonucleic Acid and Phosphoprotein in Animal Tissues, J. Biol. Chem. **161**:83-89, 1945.

9. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**:375-400, 1925.

cell counts carried out on stained films[10] of bone marrow identical with the sample subjected to chemical analysis have also been included. Both erythroblastic and myelogenous cells were counted because both share in the delayed maturation phenomena occurring in pernicious anemia, but only the counts on the erythroblastic series have been recorded here. For simplification of interpretation and enumeration of these cells so widely divergent in size and structure, a division into four groups, similar to that described by Davidson and co-workers[11] was made. In Group 1 were included deeply basophilic immature erythroblastic cells with a diameter of 18μ or more and a nucleus with a diameter of 14μ and more. The nucleus possessed a finely reticular structure. Nucleoli were occasionally present. Cells of this group we have arbitrarily designated as megaloblasts, when these were present, and pronormoblasts. The cells of Group 2, classified as basophilic normoblasts, had a diameter of between 14μ and 18μ and a nucleus with a diameter averaging 10.5μ. The nuclei were structurally less reticulated than those in the previous groups, and the cytoplasm was not so basophilic. The cells of group 3, designated as polychromatic normoblasts, had a diameter averaging 11μ and a nuclear diameter averaging 7.3μ. The chromatin was lumpy and clockface in texture, and the cytoplasm was polychromatic. Finally, in Group 4 the cellular and nuclear diameters averaged 9.3μ and 4.8μ respectively. The nucleus was pyknotic, and the cytoplasm appeared more or less eosinophilic because of the content of hemoglobin. In this group, according to our arbitrary classification, belonged the orthochromatic normoblasts. Considerable difficulty in accurately assigning borderline cells to Groups 3 and 4 was experienced.

RESULTS

The values of nucleic acid phosphorus obtained on samples of bone marrow from 31 patients are listed in the Table. The desoxyribonucleic acid phosphorus exhibits a wide range of values, extending from 0.40 to 2.63 $\times 10^{-9}$ mg. per cell; ribonucleic acid phosphorus values vary from 0.15 to 1.67 $\times 10^{-9}$ mg. By grouping the values of desoxyribonucleic acid phosphorus lying relatively close to one another, we have arbitrarily divided the figures obtained for this nuclear constituent into 6 series, with the following average values for desoxyribonucleic acid phosphorus and ribonucleic acid phosphorus in milligrams $\times 10^{-9}$ per cell for each of the series respectively: (1) 0.47 and 0.29, (2) 0.64 and 0.39, (3) 0.83 and 0.53, (4) 1.11 and 0.88, (5) 1.60 and 0.96, and (6) 2.46 and 1.54. It has been difficult to arrange the various data in tabular form so as to convey the most informative correlation. In the table the desoxyribonucleic acid phosphorus values are arranged in ascending sequence with paralleling values for other data. The variations of desoxyribonucleic acid phosphorus within each of the component series are in large part due to slight differences in technique. The cellular composition of the stained films of bone marrow from different patients varies and when expressed in percentile distribution within the erythroblastic Groups 1, 2, 3, and 4, can be separated roughly into two sections. In the first of these sections (Groups 1 and 2), the immature basophilic erythroblasts predominate; in the second section (Groups 3 and 4), the maturer normoblasts predominate, and the constituents of Groups 1 and 2 are considerably reduced. As already noted, the cells composing these two sections represent, in general, two diametrically different processes in the development of the red blood cell of man. The first of these is associated with growth, rise in nucleic acid, and increase in the number of cells. The second is related to devolutional changes in the nucleus prior to its extrusion from the cell and synthesis of hemoglobin. The changes relating to decrease in ribonucleic acid

10. These films were lent the Hematological Department.

11. Davidson, L. S. P.; Davis, L. J., and Innes, J.: The Effect of Liver Therapy on Erythropoiesis as Observed by Serial Sternal Punctures in 12 Cases of Pernicious Anaemia, Quart. J. Med. **11**:19-27, 1942.

Patient	Age, Yr.	DNAP, Mg. × 10^{-9}/Cell	RNAP, Mg. × 10^{-9}/Cell	NAP, Mg. × 10^{-9}/Cell	Nuc. Marrow, Cells in 1,000's/ Cu. Mm.	Reticulocytes, %	Hemoglobin, Gm. %	R. B. C., Millions/ Cu. Mm.	W. B. C. in 1,000's/ Cu. Mm.	Percentile, Differential Cell Counts of Bone Marrow I	II	III	IV*	Comment
										Series 1				
E. K.	70	0.40	0.25	0.65	98	2.4	9.4	2.3	3.8	37	26	14	23	Alcoholic; chronic cholecystitis; nutritional anemia; died 2 weeks later
M. Y.	62	0.50	0.38	0.88	104	1.6	12.0	3.5	9.2	36	20	16	28	Symptoms suggested pernicious anemia
2 wk. later		0.46	0.37	0.83	100	3.4	8.0	1.9	5.8	43	28	13	16	
G. W.	58	0.52	0.15	0.67	60	1.8	12.6	3.1	6.2	30	20	30	20	Nutritional anemia
										Series 2				
I. Y.	67	0.59	0.31	0.90	50	...	12.0	3.5	4.1	05	24	12	59	Enteritis necroticans, malabsorption
P. W.	38	0.60	0.27	0.87	120	3.4	7.0	2.7	4.2	10	50	22	18	Macrocytic anemia
E. M.	47	0.65	0.60	1.25	23	...	10.0	3.4	7.7	08	10	34	48	Addison's disease; essentially normal marrow
A. C.	71	0.68	0.54	1.22	69	0.8	3.8	1.0	2.5	10	20	52	18	Moderately macrocytic hypocellular marrow
J. E.	29	0.69	0.23	0.92	52	0.8	12.4	3.9	8.0	04	12	34	50	Essentially normal marrow; dysmenorrhea
										Series 3				
D. G.	61	0.76	0.41	1.17	61	0.8	14.0	3.8	5.4	17	18	40	25	Symptoms suggested pernicious anemia; had received treatment
J. W.	24	0.76	0.52	1.28	25	0.4	13.4	4.8	8.5	17	17	25	41	Anemia of many years' duration; marrow essentially normal
A. H.	40	0.78	0.35	1.13	61	0.4	6.0	1.7	6.2	15	55	25	05	Megaloblastic anemia of pregnancy
R. B.	02	0.78	0.49	1.27	113	1.4	7.9	2.0	11.2	05	21	48	26	Macrocytic anemia of childhood
J. B.	76	0.80	0.44	1.24	99	0.8	10.4	3.9	8.0	19	50	16	15	Hemosiderin deposit in macrocytic marrow; not pernicious anemia
G. H.	70	0.81	0.52	1.33	42	0.9	9.7	1.4	10.6	11	17	34	38	Nutritional hypochromic anemia; ascites; no response to liver extract
L. B.	64	0.83	0.39	1.22	50	7.6	5.8	1.7	6.5	05	23	35	37	Duodenal ulcer with hemorrhage; transfusion
M. C.	71	0.85	0.40	1.25	81	3.4	12.3	3.1	5.8	44	21	20	15	Symptoms suggested pernicious anemia
G. M.	51	0.86	0.28	1.14	81	2.4	11.9	3.1	8.2	06	08	47	40	Hypochromic anemia
P. H.	67	0.87	0.35	1.22	54	1.5	11.3	4.6	2.2	19	21	25	35	Splenectomy previously; microcytic anemia
R. S.	48	0.95	0.33	1.28	55	...	7.6	3.9	4.9	10	15	58	17	Epithelial cysts of scalp; macrocytic anemia
G. H.	54	0.97	0.43	1.40	54	1.4	14.9	5.0	5.7	07	20	53	20	Treated pernicious anemia
										Series 4				
S. M.	76	1.05	0.93	1.98	60	3.2	9.9	3.2	4.6	09	33	18	40	Normocytic anemia; chronic pyelonephritis; right renal mass
M. R.	28	1.18	0.84	2.02	45	0.1	3.4	8.2	1.2	29	50	15	09	Megaloblastic anemia of pregnancy; pneumonia
										Series 5†				
6 patients		1.60	0.96	2.56	..	...	...	...	...	44	22	15	19	Pernicious anemia
										Series 6				
J. C.	70	2.18	1.67	3.85	140	1.0	5.4	1.3	3.2	53	25	14	08	Symptoms suggested pernicious anemia
W. M.	58	2.57	1.47	4.04	76	1.4	14.9	5.0	5.7	09	25	45	21	Macronormoblastic marrow; not pernicious anemia
C. M.	66	2.63	1.50	4.13	91	0.8	10.8	3.0	7.5	10	29	50	11	Macronormoblastic marrow; not pernicious anemia

* Different types of erythroblasts in each group are described in the text.
† Five of the patients in this series are listed in detail in Group A of the following paper. Patient A. H. of Group A has been replaced by another untreated patient with pernicious anemia.

phosphorus in preparation for the transformation of the erythroblast into a vehicle for the rapid transport of certain gases within the body have been studied in detail by Thorell.[12]

A survey of the accompanying table indicates that in 14 of the 31 patients, 60% or more, and in an additional patient 50%, of the cells contained in the bone marrow belonged in the first section (Groups 1 and 2). The diagnoses for the 15 patients and their distribution within the six different series are interesting.

Two cases of nutritional macrocytic anemias associated with malabsorption belong in Series 1; of the three cases of "suggestive" pernicious anemia before treatment, one each occurs in Series 1, 3, and 6 and the six cases definitely diagnosed as pernicious anemia constitute Series 5; one each of the two cases of megaloblastic anemia of pregnancy is contained in Series 3 and 4, and one each of the two cases of macrocytic anemia, unclassified, are listed in Series 2 and 3.

Finally, there are six cases in which the combined cells of Group 2 and 3 reach 69% or over. Four of these cases belong in Series 3 and the other two cases, with marrow diagnosed as macronormoblastic, belong in Series 6. These two macronormoblastic bone marrows are interesting because of the very high content of cellular desoxyribonucleic acid phosphorus.

The above analyses indicate that the bone marrow from 31 patients (32 samples) showed in 21 individuals (70%) an accumulation of early immature cells in which the average cellular desoxyribonucleic acid phosphorus content varied from 0.46 to 2.44 $\times$ 10^{-9} mg.

In the remaining 10 patients the marrow contains cells characteristic of Groups 3 and 4. One of these patients had pernicious anemia, and had received treatment, and two others were classed as essentially normal.

COMMENT

The erythrocyte, as already indicated, has a complicated and variable history in relation to nucleic acid content. An initial rise followed by a decline occurs in both the ribonucleic acid phosphorus and desoxyribonucleic acid phosphorus but the rate of successive alterations in each of these does not run parallel. The ribonucleic acid phosphorus rises and falls more rapidly and precipitously than does the desoxyribonucleic acid phosphorus; hence for interpretation of the significance of a given amount of nucleic acid obtained on bone marrow by chemical analysis a morphological examination is necessary. Two samples of bone marrow containing types of cells which differ a great deal morphologically may contain comparable amounts of desoxyribonucleic acid phosphorus.

Normal bone marrow, in which the majority of the constituent cells are late normoblasts, has a low chemical value for nucleic acid. The average figure of 0.67 $\times$ 10^{-9} mg., obtained by us for desoxyribonucleic acid phosphorus in two cases of normal marrow, is slightly lower than the figure 0.869 $\times$ 10^{-9} mg. reported by Davidson, Leslie and White,[13] but since the range recorded by these workers was from 0.4 to 1.5 $\times$ 10^{-9} mg., this difference may not be of real significance.

12. Thorell, B.: Studies on the Formation of Cellular Substances During Blood Cell Formation, Acta med. scandinav. (Supp. 200) **129**:1-120, 1947.

13. Davidson, J. N.; Leslie, I., and White, J. C.: Quantitative Studies on the Content of Nucleic Acids in Normal and Leukæmic Cells, from Blood and Bone Marrow, J. Path. & Bact. **63**:471-483, 1951. Footnote 4.

Our average value for nucleic acid phosphorus of 2.56×10^{-9} mg. per cell in bone marrow in six cases of moderately severe pernicious anemia agrees well with the average value of 2.59×10^{-9} mg. reported by these authors. However, our figures for desoxyribonucleic acid phosphorus are higher, namely 1.60×10^{-9} mg. as compared with 1.257×10^{-9} mg., and our values for ribonucleic acid phosphorus are correspondingly lower, namely 0.96×10^{-9} mg. as compared with 1.338×10^{-9} mg. The range for the two nucleic acids recorded by these workers are respectively 0.7 to 2.1×10^{-9} mg. and 0.46 to 2.18×10^{-9} mg. In cases of severe or relapsing pernicious anemia, values for desoxyribonucleic acid phosphorus obtained by us may reach the lower figure, the significance of which will be discussed in the following paper.

It is interesting that the value 1.60×10^{-9} mg. for desoxyribonucleic acid phosphorus (approximately 1.58×10^{-8} for desoxyribonucleic acid) per cell of bone marrow in pernicious anemia of moderate severity agrees so closely with the values, ranging from 1.49 to 1.54×10^{-8}, for desoxyribonucleic acid per nucleus of liver obtained by Rose and Schweigert [14] in their studies on the effect of vitamin B_{12} on nucleic acid metabolism of the rat.

The average of 2.60×10^{-9} mg. of desoxyribonucleic acid phosphorus per cell for two cases of macronormoblastic anemia represents the maximum amount which has been obtained by us in the erythroblast.

In the consecutive stages of the developing human erythroblast, as obtained in bone marrow of different macrocytic blood dyscrasias, our chemical analyses show a progression of values for cellular desoxyribonucleic acid phosphorus from 0.40 to 2.63×10^{-9} mg. and for ribonucleic acid phosphorus from 0.15 to 1.67×10^{-9} mg. These values do not differ too widely from the ranges of 0.7 to 2.4 for desoxyribonucleic acid phosphorus and from 0.5 to 2.7×10^{-9} mg. for ribonucleic acid phosphorus for marrows of megaloblastic anemia shown in the frequency graphs of Davidson and co-workers [4] when one considers the difficulties encountered in the analysis of such a heterogeneous tissue as bone marrow.

These values of cellular nucleic acid obtained in the developing erythroblast have been divided by us into six series, forming a progression in which the mean value of each component series is increased 30 to 50% above that of the preceding one.

SUMMARY

Chemical analysis on bone marrow aspirated from 31 patients showed values per cell of desoxyribonucleic acid phosphorus ranging from 0.40 to 2.63×10^{-9} mg. and of ribonucleic acid phosphorus from 0.15 to 1.67×10^{-9} mg. These cellular values have been divided into six discrete series in ascending sequence.

Each of the series was separable into two divisions, according to whether the majority of cells in stained films of the marrow were (*a*) immature basophilic erythroblasts (pronormoblasts and early basophilic normoblasts) or (*b*) late normoblasts (polychromatic and eosinophilic).

14. Rose, I. A., and Schweigert, B. S.: Effect of Vitamin B_{12} on Nucleic Acid Metabolism of the Rat, Proc. Soc. Exper. Biol. & Med. **79**:541-544, 1952.

The desoxyribonucleic acid phosphorus content of these two types of erythroblasts (early and late) represent two diametrically different processes in relation to nucleic acid development. The first type presents a gradual increment in desoxyribonucleic acid phosphorus, and the second shows a progressive decrease leading to extrusion of nuclear material and formation of hemoglobin.

Dr. W. H. Perry, chief, and Miss Marjorie Gray, senior technician, of the hematological laboratories of the Vancouver General Hospital, assisted in this study.

NUCLEIC ACID IN CELLS OF BONE MARROW OF PATIENTS WITH PERNICIOUS ANEMIA

Changes in Content Before and After Specific Treatment

MAUD L. MENTEN, M.D., Ph.D.
AND
MARY WILLMS, M.A.
VANCOUVER, B. C., CANADA

THE DEMONSTRATION [1] that the cellular changes characteristic of the retarded hematopoiesis in bone marrow of untreated patients with moderately severe pernicious anemia was associated with a level of nucleic acid phosphorus (NAP) averaging 2.56×10^{-9} mg. per cell in that tissue led to the inference that on therapeutically supplying deficient vitamin B_{12} not only would the bone marrow rapidly regain its normal appearance but the transformation would be accompanied by a concomitant rise in the cellular nucleic acid phosphorus. Disappearance of the early basophilic megaloblasts occurred promptly; complete reversion to marrow with cells of normal size and appearance took place less readily. Chemical restitution was also delayed and an immediate reduction in cellular nucleic acid phosphorus was the rule. The reduction was followed by a rise only after a variable length of time. This report deals with the changes in nucleic acid phosphorus following the institution of treatment and with possible underlying factors.

MATERIALS AND METHODS

All patients, with the exception of one seen at Shaughnessy Hospital, were studied at the Vancouver General Hospital. The methods employed were identical with those outlined in the preceding paper [1] except that, when possible, repeated aspirations of bone marrow were performed on each patient. The first aspiration of bone marrow was made prior to treatment and the remainder at intervals of 24 or more hours subsequent to the initiation of therapy with vitamin B_{12} or liver extract.

RESULTS

The results, presented in the Table, may be divided into two groups, A and B, depending upon the duration and course of the anemia before the admission of the patient to the hospital and upon the level of cellular nucleic acid phosphorus found on the first analysis. In Group A are included six patients whose initial cellular content of nucleic acid phosphorus in bone marrow averaged 2.55×10^{-9} mg.; in

From the British Columbia Medical Research Institute and the Vancouver General Hospital, Vancouver.

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1. Menten, M. L., and Willms, M.: Changing Values of Nucleic Acid of Cells in Bone Marrow During the Development of the Red Blood Cell, A. M. A. Arch. Path., this issue, p. 343.

Changes in Bone Marrow Cells from Patients with Pernicious Anemia Before and During Treatment

Patient	Age, Yr.	Days of Treatment	RNAP, Mg. × 10^{-9}/Cell	DNAP, Mg. × 10^{-9}/Cell	NAP, Mg. × 10^{-9}/Cell	Marrow Nuc. Cells, 1,000's/Cu. Mm.	Ratio of DNAP to RNAP	Reticulocytes, %	Hemoglobin, Gm./100 Cc.	R. B. C., Millions/Cu. Mm.	W. B. C., 1,000's/Cu. Mm.	Differential Cell Counts on Bone Marrow Films; Relative Percentages of Different Types of Erythroblasts Before and with Treatment I	II	III	IV	Treatment
Group A																
J. A.	85	0	0.95	1.51	2.46	60	1.6	2.0	3.6	0.9	2.2	42	24	20	14	15 hr. after first sternal puncture, liver extract of 30 units; then daily, 30 units
		1	1.24	1.27	2.51	100	1.0	3.0	3.6	0.9	2.2	40	31	17	12	
		2	0.42	0.80	1.22	91	2.0	4.8	...	...	...	12	55	16	17	
		3	0.47	1.31	1.78	99	2.8	5.8	...	...	...	10	20	55	15	
A. B.	78	0	0.97	1.54	2.51	70	1.6	0.6	6.6	1.7	4.1	48	17	05	30	Folic acid, 30 units daily
		3	0.45	1.32	1.77	60	3.0	2.5	2.5	...	6.6	07	26	47	20	2d day, 500 cc. transfusion; vitamin B_{12}, 30 mg. 3d day, vitamin B_{12}, 3 mg.
A. C.	46	0	0.97	1.54	2.51	81	1.6	3.0	14.0	3.9	7.3	43	24	20	13	2d day, liver extract, 15 units; this dosage repeated twice weekly
		23	0.45	1.32	1.77	144	3.0	3.6	15.2	4.5	4.5	15	22	26	37	
A. H.	63	0	0.67	1.96	2.63	141	2.9	2.6	5.5	2.1	5.1	22	18	09	51	.....
H. H.	68	0	0.67	1.80	2.47	30	2.7	1.5	9.2	2.2	3.3	13	25	42	20	On admission and daily, 5 mg. ascorbic acid; on 7th day, vitamin B_{12}, 30 mg., and then repeated every third day
		9	0.46	1.38	1.84	58	3.0	6.7	...	...	3.0	05	15	50	30	
		11	0.31	0.56	0.87	85	1.8	9.4	...	...	...	05	12	45	38	
		17	0.20	0.62	0.82	58	3.1	10.0	...	...	...	09	08	53	30	
		53	0.35	0.83	1.18	102	2.4	1.0	13.3	4.1	2.8	..	..	..	..	
A. W.	74	0	1.24	1.46	2.70	55	1.2	0.8	4.9	1.2	1.8	46	14	15	25	On day following sternal puncture, liver extract, 4 units, and then repeated daily
		4	0.27	0.43	0.70	85	1.6	1.5	4.7	...	...	05	20	55	20	
Group B																
W. M.	65	0	0.43	0.83	1.26	72	1.9	7.4	7.7	2.5	5.2	27	40	28	05	Liver extract, 15 units for the 11-day period
		11	0.38	1.63	2.01	75	4.3	1.0	8.8	2.6	...	18	35	20	27	
C. S.	75	0	0.38	0.97	1.33	65	2.6	1.6	7.3	1.8	6.0	25	11	05	59	Shortly before first sternal puncture, vitamin B_{12}, 5 mg., then repeated daily for 16 days; then vitamin B_{12}, 20 mg. twice weekly; on 6th day, folic acid, 30 mg. daily
		2	0.37	0.49	0.86	30	1.3	1.8	...	1.3	...	07	10	48	35	
		5	0.34	0.54	0.88	80	1.6	10.1	...	...	...	05	22	50	23	
		7	0.26	0.53	0.79	65	2.0	14.4	8.0	2.0	...	02	25	55	18	
		28	0.73	2.15	2.88	46	3.0	1.3	12.2	3.4	...	..	..	..	..	
J. R	80	0	0.70	0.86	1.56	112	1.2	1.4	4.7	1.3	2.9	56	10	16	18	On second day after first sternal puncture, liver extract, 45 units, and repeated daily for 1 week; then 30 units twice a week; also on second day, ascorbic acid, 250 mg. daily and multivitamins, 2 capsules twice daily.
		3	0.60	0.67	1.27	60	1.1	1.4	4.9	1.2	4.7	32	16	36	16	
		4	0.50	0.77	1.27	139	1.5	5.0	...	...	...	07	24	55	14	
		6	0.10	0.30	0.40	101	3.0	25.9	5.4	1.6	4.7	05	15	70	10	
		11	0.25	0.88	1.13	120	3.5	8.8	6.3	1.7	7.0	..	..	..	..	
K. K.	24	0	0.47	0.72	1.19	64	1.5	1.2	4.7	1.2	3.6	48	15	28	09	After first sternal puncture, vitamin B_{12}, 15 mg., then twice weekly; on 10th day also ascorbic acid, 100 mg. q.3.h.; multivitamin capsules 1 q.3.h.
		4	0.33	0.90	1.23	58	2.7	20.2	...	...	...	05	30	52	13	
		10	0.33	0.86	1.19	43	2.6	38.0	...	...	...	02	20	71	07	
		22	0.34	0.84	1.18	45	2.5	....	8.3	2.3	5.7	04	12	73	11	
R. C.	43	0	0.30	0.50	0.80	94	1.7	0.4	4.6	1.6	2.2	50	20	15	15	First day, liver extract, 15 units, and repeated daily
		11	0.35	0.52	0.87	59	1.5	...	...	...	...	02	12	48	38	
		24	0.27	0.57	0.84	51	2.1	3.9	11.9	3.4	6.7	05	10	68	17	

Group B are listed five patients whose nucleic acid phosphorus content on first analysis was approximately 0.80, 1.20, or 1.56 $\times$ 10^{-9} mg. per cell. The patients included in Group A, of whom five were seen by us for the first time, showed generalized symptoms of only moderate severity. Those recorded in Group B either had had intermittent and inadequate treatment over a variable time and were in relapse or were manifesting symptoms and anemia of considerable severity when the first bone marrow aspiration was made. Besides the results of chemical analyses, included in the Table are the percentages of the bone-marrow erythroblasts in Groups 1, 2, 3, and 4, as classified in the preceding paper. Also tabulated are data on reticulocytes, red and white blood cells, and hemoglobin.

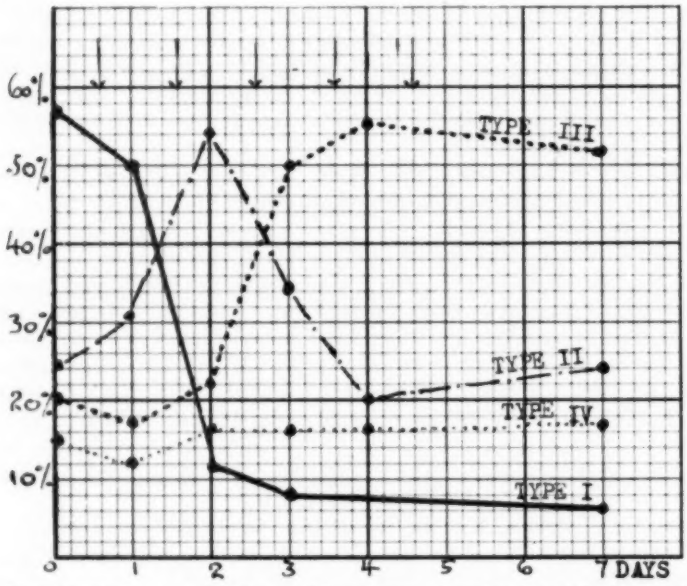
Although patients of the two groups responded to liver extract or vitamin B_{12} therapy by an immediate fall in both ribonucleic acid phosphorus and desoxyribonucleic acid phosphorus, they differed in the speed with which they retrieved the loss. Patients of Group B generally showed a slower reparative response. Patients of Group A usually recovered more promptly from the primary depressing effects of specific treatment, and at the end of 72 hours the first three patients listed in this group were commencing to show an upward trend in cellular desoxyribonucleic acid phosphorus.

Changes in the nucleic acid content of the bone marrow induced by specific therapy in untreated patients with pernicious anemia of moderate degree are illustrated in the figures shown for patient A. J., who received the first injection of liver extract 15 hours after the initial aspiration of bone marrow. The second sample of bone marrow, withdrawn nine hours later, showed only a small increase in ribonucleic acid phosphorus with a corresponding decrease in desoxyribonucleic acid phosphorus. Twenty-four hours after the second injection of liver extract (and 33 hours after the first injection) there was a marked fall in nucleic acid phosphorus, a composite of 35% ribonucleic acid phosphorus and 65% desoxyribonucleic acid phosphorus. Continuation of therapy produced during the next 24 hours a rise in the nucleic acid phosphorus due mainly to increase in desoxyribonucleic acid phosphorus. Similar changes in nucleic acid phosphorus values were found for Patient A. B. before and after 72 hours of treatment.

Variation in the ribonucleic acid phosphorus : desoxyribonucleic acid phosphorus ratio was a reflection of the difference in the reaction of the two integrant nucleic acids to treatment. Liver extract or vitamin B_{12} invariably augmented the ratio from between 1 : 1 and 1 : 1.6 in untreated patients to 1 : 2 and above after treatment. High initial values of this ratio in Patients A. H. and H. H., we believe, indicated previous treatment. W. M. and C. S. of Group B, with a similar high initial ratio, were known to have received treatment of varying efficacy for a considerable period before admission to the hospital.

The pathologic effects resulting from deficiency of the anti-pernicious-anemia principle were more pronounced in patients of Group B, as evidenced by a lower initial value of cellular nucleic acid phosphorus and especially desoxyribonucleic acid phosphorus. An extremely low content of nucleic acid phosphorus, reaching 0.80 $\times$ 10^{-9} mg., with desoxyribonucleic acid phosphorus of 0.52 $\times$ 10^{-9} mg. per cell, was found in Patient R. C. It may be added that "personality" changes, probably ascribable to the disease per se, were present in this patient before treatment, and disappeared with treatment. Although treatment of the patients in Group

B evoked a train of changes comparable qualitatively to those described for Group A, the immediate depressing effects on the cellular nucleic acid phosphorus level were prolonged from 11 to 30 days, as is shown by values recorded for Patients J. R. and K. K. Indeed, the former patient died before the upward trend of reparative synthesis of nucleic acid phosphorus, and especially of the desoxyribonucleic acid phosphorus component, occurred. It is not clear in this patient whether the very low values of 0.40×10^{-9} mg. per cell for nucleic acid phosphorus on the twentieth day was solely the result of response to vitamin B_{12} or whether the meralluride (mercuhydrin®), which was injected intramuscularly for a nephrotic condition a few hours before the aspiration of the sample of bone marrow, was a factor in its production.



Percentile changes in distribution of different types of erythroblasts following specific treatment. Arrow indicates injection of liver extract.

The accessory medicaments of folic acid in patients A. B., C. S., and H. H. seemed to have exerted little or no accelerating influence on the reparative effect of vitamin B_{12}. Also, the addition of ascorbic acid to the regimen of H. H. and J. R., as well as the addition of "multivitamins" to that of the former patient, gave little appreciable evidence of speeding up the return to normal bone marrow.

Although a general relationship was noted between the chemical findings and associated anemia and leucopenia, no definite proportionality could be established. In the marrow of untreated patients the only clear-cut correlation that could be discerned between the level of nucleic acid phosphorus and distribution of cells in stained films within the four groups (1, 2, 3, and 4) was a preponderance of

basophilic megaloblasts in Group 1. Treatment induced a rapid disappearance of these cells and a gradual accumulation of the polychromatic normoblasts in Group 3. This finding is in agreement with that reported by Davidson and co-workers.[2] Our graph (Chart) showing the influence of vitamin B_{12} or liver-extract therapy on the redistribution of types of cells in bone marrow is similar to that recorded by these workers, although our observations cover a longer period following treatment. In addition, a rapid accumulation of cells in Group 4 instead of Group 3 was noted in Patient C. S. on the resumption of medication after a lapse of many months. Of interest is the finding that the number of reticulocytes reached a peak coincident with the highest rise in percentage of polychromatic normoblasts of Group 3. Both reactions, furthermore, are related to a low "pretreatment" value of nucleic acid phosphorus and a prolonged period preceding the upward restitutive response following treatment, as seen in Patients J. R. and K. K.

COMMENT

Although only a small number of patients with pernicious anemia have been studied by us, certain findings seem worthy of note. The first is that lack of the anti-pernicious-anemia principle (vitamin B_{12}) which results in retarded hematopoiesis and delayed maturation of erythroblastic and myelogenous cells of the bone marrow was associated with a stationary level of nucleic acid phosphorus in that tissue. The nucleic acid phosphorus was made up of high ribonucleic acid phosphorus and low desoxyribonucleic acid phosphorus; inferentially, the low desoxyribonucleic acid phosphorus was due to an arrest in its formation. The extent of this arrest depended apparently upon the degree and duration of the vitamin deficiency. Patients in relapse or exhibiting severe symptoms (Group B) showed a lower average initial value of nucleic acid phosphorus, namely, 0.80, 1.23, or 1.56×10^{-9} mg. and corresponding desoxyribonucleic acid phosphorus of 0.50, 0.84 and 0.86×10^{-9} mg., than those in the more moderate stages of the disease (Group A) with an average initial level of nucleic acid phosphorus of 2.55×10^{-9} mg. and desoxyribonucleic acid phosphorus of 1.63×10^{-9} mg. per cell. It may be pointed out that the first two figures, namely 0.80 and 1.23×10^{-9} mg. for nucleic acid phosphorus per cell correspond fairly closely with the mean values of the two contiguously lying Series 2 and 3 found by us for the developing erythrocyte.[1] The third figure of 1.56×10^{-9} mg. is somewhat lower than the average 2.00×10^{-9} mg. of Series 4. The question may be raised as to the accuracy of these figures for a tissue with such a mixed population of cells as bone marrow. That the heterogeneity of normal bone marrow differs from that occurring in the condition of arrested cellular maturation obtaining in pernicious anemia is readily recognized morphologically. The essential identity of our mean value for nucleic acid phosphorus of 2.55×10^{-9} mg. per cell in six cases of pernicious anemia of moderate degree, with the mean value of 2.59×10^{-9} mg. reported by Davidson and co-workers[3] in 12 cases, lends support to attributing a fair degree of accuracy to this figure.

2. Davidson, L. S. P.; Davis, L. J., and Innes, J.: The Effect of Liver Therapy on Erythropoiesis as Observed by Serial Sternal Punctures in 12 Cases of Pernicious Anemia, Quart. J. Med. **11**:19-27, 1942.

3. Davidson, J. N.; Leslie, J., and White, J. C.: Nucleic Acid Content of the Cell, Lancet **1**:1287-1290, 1951.

Our mean value of 1.60×10^{-9} mg. for desoxyribonucleic acid phosphorus, or 1.58×10^{-8} mg. for desoxyribonucleic acid, per cell of bone marrow is in close agreement with the mean values of 1.49 and 1.54×10^{-8} mg. of desoxyribonucleic acid per nucleus of the hepatic cell of rats, on a diet with or without vitamin B_{12} reported by Rose and Schweigert.[4] These authors are inclined to attribute the depressing effect of lack of vitamin B_{12} on metabolism of nucleic acid to decrease in cell division rather than to reduction in the amount of nucleic acid per cell. However, their experiments were not carried on longer than four weeks.

Our studies of stained films of bone marrow of pernicious anemia indicate that arrest in the cellular maturation at the higher level of nucleic acid phosphorus differs from that occurring at the lower levels, not so much in increased numbers of early immature cells as in the degree of their qualitative change. Increase in the size of the cells, hyperhemoglobinization, and variations in structure of the nucleus became pronounced as the condition worsened. Whether the lower "pretreatment" values of nucleic acid phosphorus indicate a greater retardation of hematopoiesis provoked solely by prolonged and exaggerated lack of vitamin B_{12} or whether deficiency of other chemical substances is associated in the production of this retardation cannot at present be established. That folic acid, vitamin C, and "multivitamins" are not important factors in enhancing the reparative action of vitamin B_{12}, is indicated by our analyses and supports the recent clinical report of Chodos and Ross [5] of the inadequacy of folic acid in the treatment of pernicious anemia. Regardless of the initial level of nucleic acid phosphorus, the primary decrease in response to vitamin B_{12} therapy is in agreement with that also reported, but without accompanying figures, by Davidson and associates.[3] Although an immediate decline occurs in both types of nucleic acid, the most marked fall takes place in ribonucleic acid phosphorus, and this reduction parallels the speedy disappearance from the marrow of the basophilic megaloblasts (Group I) which is succeeded by a gradual rise in the polychromatic normoblasts (Group III). The loss of the megaloblasts from the bone marrow occurring within 24 hours after the administration of folic acid is interpreted by Frühling and co-workers [6] as direct lysis of these cells, because they found during this period a coincident rise in uric acid of the blood and urine without an analogous rise in the number of red blood cells.

The decline in the content of nucleic acid following treatment is temporary; its duration varies from about 3 to 30 days and is roughly proportional to the degree of reduction of the initial value of the marrow in untreated pernicious anemia. A further index of the duration of the period of decline is the height of response of the reticulocytes, and the accompanying rise in the percentage of polychromatic normoblasts. In this study as in the previous one on blood dyscrasias,

4. Rose, I. A., and Schweigert, B. S.: Effect of Vitamin B_{12} on Nucleic Acid Metabolism of the Rat, Proc. Soc. Exper. Biol. & Med. **79**:541-544, 1952.

5. Chodos, R. B., and Ross, J. F.: The Effect of Combined Folic Acid and Liver Extract Therapy, Blood **6**:1213-1233, 1951.

6. Frühling, L.; Roger, S., and Spenler, H.: Contribution à l'étude de la physiopathologie de la moelle osseuse dans la maladie de Biermer: Le problème du mégaloblaste, Sang **20**:164-195, 1949. Frühling, L.; Mandel, P.; Metais, P., and Moise, R.: Sur certains aspects du catabolisme nucléique endogène dans la maladie de Biermer traitée par l'acide folique: Contribution à l'étude du mégaloblaste, Sang **21**:541-548, 1950.

two samples of bone marrow with dissimilar types of cells may show the same chemical value for nucleic acid; one of the samples contains the early immature cells of retarded hematopoiesis and arrested formation of desoxyribonucleic acid, the other is made up of late, mature normoblasts with a diminishing content of this nucleic acid. Many of the cells in the latter category show in Giemsa-stained films loss of detail and a homogenous pink appearance of the nuclei, characteristic of early degenerative changes. Leuchtenberger [7] has shown on pyknotic nuclei, with Feulgen and with pyronin-methyl green stains, that such nuclear degenerations are related to depolymerization and loss of nucleic acid. This finding has been substantiated by Korson.[8]

The different rates of decrease and increase of desoxyribonucleic acid and ribonucleic acid in both the "treatment" and "pretreatment" periods make it difficult to assess chemically the direct effect of vitamin B_{12} on each of these two cellular constituents but do seem to indicate their independent synthesis and activity. Whether the change in desoxyribonucleic acid phosphorus, with concomitant alterations in the morphologic structure of the nucleus, reflects the basic activity of the vitamin B_{12} and conditions of the ribonucleic acid phosphorus content and related basophilia of the cytoplasm, or whether vitamin B_{12} simultaneously induces changes in both ribonucleic acid and desoxyribonucleic acid directly, can be established only by further studies. Data on this phase of the problem might aid in the elucidation of the different chemical activities of vitamin B_{12}, which seems to be necessary for the developmental consummation of other cytoplasmic constituents of the erythrocyte, such as stroma, etioporphyrin, and hemoglobin, as well as maturation processes occurring in the cells of the granulocytic series. That vitamin B_{12} contributes, directly or indirectly, to the formation of stroma and envelope of the erythrocyte is indicated by the shortened life of red blood cells in pernicious anemia from 120 days to 72 and 60 days [9] and 52 days.[10] Relation to hemoglobin and its precursors is evidenced by the formation of coproporphyrin of isomer type III in direct proportion to the number of reticulocytes recently demonstrated by Schwartz and Wikoff [11] in the blood of pernicious anemia and other hematologic conditions in which reticulocytes are formed. Also, that the role of vitamin B_{12} in developing and maintaining the cell is not limited to the erythrocytic and granulocytic series of bone marrow but is concerned with maintenance of the integrity of the cells of the nervous system and gastrointestinal tract is attested by the lesions which may occur in these tissues in pernicious anemia. One difficulty in the interpretation of the role played by vitamin B_{12} in the synthesis of the nucleic acids is that it apparently acts as a co-enzyme, and substances with which it combines are not known.

7. Leuchtenberger, C.: A Cytochemical Study of Pyknotic Nuclear Degeneration, Chromosome **3**:449-473, 1949.

8. Korson, R. A.: Microspectrophotometric Study of Red Cell Nuclei During Pyknosis, J. Exper. Med. **93**:121-128, 1951.

9. Callender, S. J.; Loutit, J. F., and Jope, E. M.: Discussion on Life and Death of the Red Blood Corpuscle, Proc. Roy. Soc. Med. **39**:755-762, 1946.

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11. Schwartz, S., and Wikoff, H. M.: The Relation of Erythrocyte Coproporphyrin and Protoporphyrin to Erythropoiesis, J. Biol. Chem. **194**:563-573, 1952.

SUMMARY

The desoxyribonucleic acid phosphorus content per cell of bone marrow aspirated from patients with pernicious anemia ranged from 1.80 to 0.50 mg. $\times 10^{-9}$. The ribonucleic acid phosphorus varied from 1.24 to 0.30 mg. $\times 10^{-9}$. The higher values were obtained in patients with anemia and symptoms of moderate severity; the lower values were found in patients in relapse or with inadequate treatment over a variable time.

The nucleic acid values tended to fall into groups similar to those reported by us for blood dyscrasias with delayed maturation in hematopoiesis.

Specific treatment caused in the bone marrow a rapid disappearance of megaloblasts with an increase in polychromatic erythroblasts and a decline in cellular nucleic acid lasting from 3 to 30 days. The duration of the decline was proportional to the degree of reduction in the initial values of cellular nucleic acid, percentile rise in polychromatic erythroblasts, and the height of the response of reticulocytes.

Dr. W. H. Perry, chief, and Miss Marjorie Gray, senior technician, of the department of hematology of the Vancouver General Hospital, assisted in this study. Dr. Viola Rae, pathologist, Shaughnessy Hospital, supplied clinical material.

AMYLOID LOCALIZED TO THE HEART

Analysis of Twenty-Nine Cases

ALBERT J. JOSSELSON, M.D.
RAYMOND D. PRUITT, M.D.
AND
JESSE E. EDWARDS, M.D.
ROCHESTER, MINN.

AS JUDGED from the literature, amyloid deposition restricted largely to the heart is uncommon, although cardiac involvement in primary systemic amyloidosis and in amyloid disease complicating multiple myeloma is recognized as a common manifestation of the latter conditions. In secondary amyloidosis, also, myocardial involvement may occur.

Steinhaus [1] in 1902 described a case of amyloid disease limited to the heart. Since that time other reports [2] dealing with amyloid disease restricted to the heart have occasionally appeared. Although the dominant feature of the cases reported by Hulbert and Meyer,[3] and Kerwin,[4] and Larsen [5] was amyloid disease of the heart, postmortem examination revealed deposits of amyloid in other tissues, presenting a distribution and degree of involvement usually seen in primary systemic and secondary amyloidosis.

Fellow in Medicine, Mayo Foundation (Dr. Josselson); from the Division of Medicine (Dr. Pruitt) and the Section of Pathologic Anatomy (Dr. Edwards), Mayo Clinic.

Abridgment of portion of thesis submitted by Dr. Josselson to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

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3. Hulbert, B., and Meyer, H. M.: Primary Amyloidosis of the Heart, Am. Heart J. **38**:604 (Oct.) 1949.

4. Kerwin, A. J.: Idiopathic Amyloid Disease of the Heart, J. Lab. & Clin. Med. **22**:255 (Dec.) 1936.

5. Larsen, R. M.: Pathological Study of Primary Myocardial Amyloidosis, Am. J. Path. **6**:147 (March) 1930.

In 1948, King [6] reported a series of five cases of amyloid disease restricted to the heart which he encountered in a relatively short time in his own pathologic material.

In 1949, Dahlin and one of us (Edwards [7]) reported 13 cases of amyloid deposition limited to the heart. These cases included five encountered in a survey of 100 hearts obtained consecutively from men aged 80 to 89 years at the time of death. The remaining eight cases were encountered among routine necropsies.

The current work was undertaken to study the incidence and functional significance of amyloid deposition restricted largely or entirely to the heart. The reports of King [6] and of the earlier study from the Mayo Clinic revealed that the incidence of this condition of the heart was surprisingly high and that the cases in which this entity was observed represented patients in the older age groups. Accordingly, it was decided to study a larger group of patients to determine the incidence of the condition in several of the older decades of life. Included in this survey are the 13 cases previously reported by Dahlin and Edwards, the cases encountered in a larger survey, and those that were observed in routine necropsies during the period of this study.

The data obtained in the present investigation reveal that this entity is not nearly as uncommon as would be supposed from the paucity of the reports in the literature, and it is probable that some cases go unrecognized.

MATERIAL AND METHOD

Data on 29 cases were collected for this analysis. Thirteen of the cases were those of Dahlin and Edwards [7] (Cases 6 to 18 of this study). (These included five cases found in a survey of the hearts of 100 men in the ninth decade of life. The other eight cases were encountered in routine necropsy material during the period of that study, which covered about two years.) Data on a second group, of 11 cases (Cases 19 to 29), were collected from routine necropsy material in the 32-month period following the previous study. A third group, of five more cases (Cases 1 to 5), was discovered in a survey of all routine histologic sections of the heart which had been taken at the time of necropsy in 500 additional cases in the seventh, eighth, and ninth decades of life. The 500 additional cases represented 100 men and 100 women each in the seventh and eighth decades and 100 women in the ninth decade. The 100 hearts examined by Dahlin and Edwards from men in the ninth decade were considered a pilot study of this one and were added to the 500 of the more recent study. In summary, 600 hearts were surveyed from 100 men and 100 women, each in the seventh, eighth, and ninth decades.

The number of available sections of heart for the initial survey varied from one to eight per case. In most instances there were two sections, one taken from the lateral aspect of the left ventricle and one from the ventricular septum. The sections had been stained with hematoxylin and eosin. With experience, deposits of amyloid could be distinguished from scar tissue with reasonable accuracy in sections stained with hematoxylin and eosin. While the two substances had the same color, as a rule, amyloid at times stained with a faint bluish tint. More reliable as a distinguishing method was to observe the texture of the substance when the shutter of the microscope condenser was placed in an almost closed position. In these circumstances, collagen clearly appeared to have a fibrillar character, while amyloid was amorphous.

In cases in which the results of examination of the sections stained with hematoxylin and eosin were considered positive for amyloid, confirmation of the presence of amyloid in each case was sought by the use of methyl violet stain for amyloid. Usually the evidence afforded

6. King, L. S.: Atypical Amyloid Disease, with Observations on a New Silver Stain for Amyloid, abstracted, Am. J. Path. **24**:671 (May) 1948; Atypical Amyloid Disease, with Observations on a New Silver Stain for Amyloid, ibid. **24**:1095 (Sept.) 1948.

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by the special stain was confirmatory in nature. In a few instances the results obtained by the special stain did not support the presence of amyloid. Such cases were designated as negative for amyloid. In addition to the stains mentioned, Verhoeff's elastic-tissue stain counterstained with Van Gieson's connective-tissue stain, the Van Gieson connective-tissue stain alone, or Mallory's phosphotungstic acid hematoxylin stain as modified by Lieb[8] was employed on selected sections. Each of these stains was found exceedingly useful. Sections stained with Van Gieson's connective-tissue stain were reliable in distinguishing masses of amyloid from bundles of collagen. Amyloid stains yellow with the Van Gieson method, while bundles of collagen stain red. With Mallory's phosphotungstic acid hematoxylin stain, muscle fibers stain purple; collagen, port-wine red, and amyloid, brownish red. The contrast in color between that of the muscle fibers and that of amyloid made the latter stain particularly useful in obtaining photomicrographs of lesions known to represent amyloid infiltration.

Since all the gross specimens of hearts were available, these were restudied in the cases in which amyloid was found to be present, and from each specimen additional sections were taken, making available a section of the wall of each chamber, a section of the ventricular septum, and a section of the endocardium of each atrium. In 11 positive cases, a section was also prepared from the left auricular appendage.

OBSERVATIONS

It was observed in the pilot study that deposits of amyloid in the heart might be identified on gross inspection without special preparation of the heart.[7] In the atrial endocardium these deposits consisted of tiny translucent gray to pink elevations, measuring from pinpoint size to approximately 0.5 mm. in diameter. Such lesions appeared either in localized collections or were diffusely deposited. In some instances there were conglomerations of these endocardial deposits (Fig. 1).[9]

Reinspection of the endocardial surfaces of the atria of all the hearts positive for amyloid in the series revealed deposits which were recognizable grossly in 22 of the 29 cases. The deposits of amyloid noted grossly in the atrial endocardium were represented on microscopic examination as discrete masses of the material separating the collagenous and elastic fibers of the endocardium (Fig. 1*d*). While involvement of the atrial endocardium formed a striking gross and microscopic feature, the ventricular endocardium was usually affected to only a minor degree, if at all. Although involvement of the atrial endocardium in itself probably was of no functional consequence, its recognition usually could be taken as an index of myocardial involvement.

In the myocardium, the initial site of deposition of amyloid was closely related to the reticulum fibers beside the muscle cells, that is, between the muscle fibers and the adjacent capillaries. As the deposition increased in amount, the muscle fibers became cystic or atrophic and finally might disappear entirely, the amyloid coalescing to form a nodule with no, or only atrophic, muscle fibers (Fig. 2). In the great majority of the cases studied the deposits of amyloid were in scattered foci (Fig. 3). The largest of these deposits measured about 1 mm. in diameter.

In the majority of the cases in which amyloid was related to the myocardial fibers, the walls of the myocardial blood vessels were also involved. In the survey, several cases also were encountered in which amyloid was found only in the walls of the coronary vessels and not among the muscle fibers. Such cases were not included in this study as "positive" cases, however.

8. Lieb, E.: Modified Phosphotungstic Acid-Hematoxylin stain, Arch. Path. **45**:559 (April) 1948.

9. The paper dealing with the electrocardiographic features of certain of the cases observed in this study maintains the same case numbers as this paper.

The degree of amyloid infiltration of the walls of the several chambers was graded 1 to 4 on the basis of the size and distribution of the deposits in the myocardium. If, in representative sections of a given chamber, an average of one focus of amyloid deposition was found per low-power field, the amyloid in the wall of that chamber was designated as Grade 1. By a similar method of grading, sections with two foci per low-power field were graded 2, those with three were graded 3,

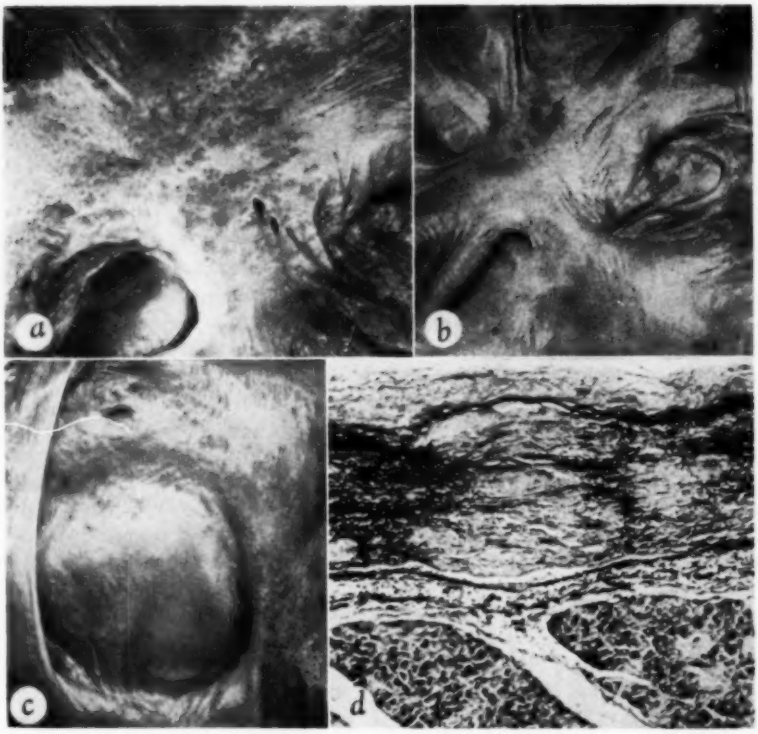

Fig. 1.—(*a*) Right atrium. Conglomerate lesions of amyloid deposition in the endocardium from a woman 88 years old dying of congestive cardiac failure. (*b*) Left atrium. A small collection of discrete nodules of amyloid involving the endocardium from a man 84 years of age dying of congestive cardiac failure. (*c*) Right side of the atrial septum and valve of the inferior vena cava. Many lesions of amyloid infiltration of the endocardium are present in the floor and in the limbus of the fossa ovalis. (*d*) Section of the left atrial endocardium and underlying myocardium. In the endocardium, corresponding with a lesion observed grossly, there is heavy deposition of amyloid which separates and interrupts elastic fibers (Verhoeff's elastic-tissue stain counterstained with Van Gieson's connective-tissue stain; × 70). Illustrations *c* and *d* are from a 75-year-old man with congestive cardiac failure.

and those with four or more were graded 4 (Fig. 3). The amyloid deposition in the entire specimen of heart was then rated as "mild," "moderate," or "severe" on the basis of a composite of the ratings of the individual sites.

In the majority of instances the degrees of amyloid infiltration were approximately the same in the various sites studied in a given case. Except in the cases

in which the degree of amyloid deposition was classified as mild, each portion of the heart studied was found involved. In the cases of mild deposition some of the areas were without recognizable amyloid, while its presence was evident in other locations. In one case (Case 17), the involvement of the left ventricular myocardium and the atrial endocardium was graded 4, while no amyloid could be identified in the sections of the ventricular septum or in the myocardium of either atrium; involvement of the right ventricular myocardium was graded 2. In such a case the degree of deposition was classified "moderate." Of the 29 cases, in 8 the condition was graded "mild," in 8 "moderate," and in 13 "severe."

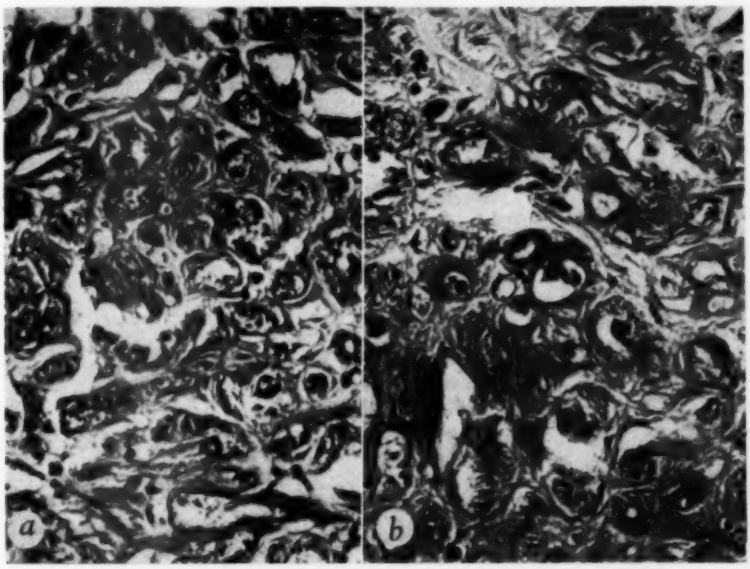

Fig. 2.—Sections from the left ventricular myocardium of a 75-year-old man with a moderate degree of amyloid deposition in the heart (hematoxylin and eosin; ×400). Death resulted from carcinomatosis secondary to carcinoma of the urinary bladder. (*a*) The myocardial fibers are surrounded by reticulum fibers thickened by the deposition of amyloid. Some muscle fibers have a cystic appearance contributing to the formation of "amyloid rings." (*b*) The amount of amyloid is greater than that illustrated in *a*. Loss of muscle fibers and coalescence of amyloid deposits are evident.

In the 29 cases the only organ that consistently contained deposits of amyloid was the heart. In the course of the study, the sections of all the organs in these 29 cases were reviewed in a further search for deposits of amyloid. Minute amounts were found in 11 cases. The structures so involved included pulmonary alveolar septa, pulmonary vessels, periadrenal blood vessels, and small pancreatic and renal blood vessels. In contrast with these findings, the features of primary systemic amyloid disease usually include cardiac involvement in addition to significant amounts of amyloid in various other tissues. In the 29 cases under consideration

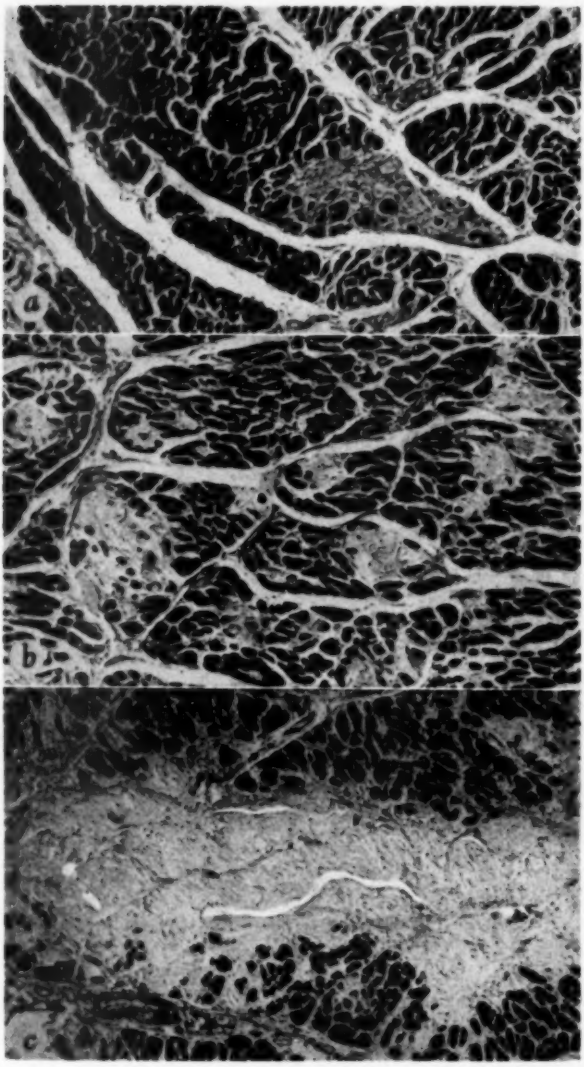

Fig. 3.—Sections of the left ventricular myocardium stained with Mallory's phosphotungstic acid hematoxylin (×70). (*a*) Occasional focus of amyloid replacing muscle fibers. The patient was an 84-year-old woman in whom the degree of amyloid involvement of the heart was graded "mild." Death resulted from perforation of a gastric ulcer and peritonitis. (*b*) Many foci of amyloid replacement of muscle. (*c*) Same case as illustrated in *b*. A conglomerate mass of amyloid replaces muscle fibers. The patient was a 75-year-old man with amyloid involvement of the heart graded "severe." Death resulted from congestive cardiac failure. Other illustrations from the same case appear in Figure 1*c* and *d*.

here, the extracardiac amyloid was so insignificant in amount as to make it sufficient, for practical purposes, to consider that the deposition of amyloid was limited to the heart.

Among the 600 hearts considered in our study, the incidence of amyloid deposition restricted to the heart is recorded in the Table according to age and sex of the patients. No case of this condition was encountered either in men or in women of the seventh decade of life, all the 10 cases representing older patients. The incidence was somewhat higher in the ninth than in the eighth decade. Among the 19 cases which were not part of those encountered in the survey of 600 hearts but in which amyloid deposition restricted to the heart was observed as part of routine necropsy work, only three patients were found to be less than 70 years of age, their ages being 63, 65, and 68 years, respectively. The average age in the 29 cases was 82 years.

To test further the relation of age to the development of amyloid deposition restricted to the heart, the average age of the patients was determined in each of

Incidence of Amyloid Deposition Restricted to the Heart in a Sample of 600 Cases Surveyed, According to Age and Sex

Age, Yr.	Cases of Amyloid Deposition in the Heart per 100 Men and 100 Women in Each Decade		
	Men	Women	Total
60-69	0	0	0
70-79	3	0	3
80-89	5	2	7
Total	8	2	10

the three categories of deposition labeled "mild," "moderate," or "severe." Of the eight patients designated as having mild forms of the condition, the ages ranged from 63 to 89 years, the average being 76.6 years. The average age of the eight patients with moderate involvement was 80.8 years, the range being from 75 to 90 years. Of the 13 patients with severe involvement of the heart the average age was 85.5 years, the range being from 75 to 100 years.

There were 21 men and 8 women in the series. This figure should be interpreted, however, with knowledge of the fact that the over-all ratio of males to females encountered at necropsy at the Mayo Clinic is approximately 8:5. When equal numbers of men and women of corresponding ages were compared, the ratio of men to women who had amyloid deposition limited to the heart was 4:1. The majority of the 29 patients were underweight, on the basis of comparison with average weights for adults of the age groups studied; 13 of 19 men and 6 of 8 women were 10 lb. (4.5 kg.) or more underweight. No data were available on the weights of the other two patients.

The incidence of malignant lesions in the cases under consideration was striking. At necropsy, carcinoma was found in 12 cases, multiple myeloma in 1. Among these, there were five instances of carcinoma of the prostate, three of carcinoma of the pancreas, two of carcinoma of the sigmoid colon, and one each of carcinoma of the stomach, urinary bladder, and rectum. (One patient had both carcinoma of the sigmoid colon and carcinoma of the prostate.)

The incidence of bronchopneumonia and congestive cardiac failure was impressive, being encountered in 13 and 6 instances, respectively. Of the six cases in which congestive cardiac failure occurred, the degree of amyloid deposition in the heart was moderate in two (Cases 22 and 25), and severe in four (Cases 8, 18, 23, and 26). In one of the last-mentioned cases (Case 8) there was old myocardial infarction, and the degree of coronary atherosclerosis was severe. In none of the five other cases in which congestive cardiac failure occurred was the degree of coronary atherosclerosis observed to be greater than moderate. In these cases the blood pressure ranged from 175 mm. of mercury systolic and 78 mm. diastolic to 100 systolic and 50 diastolic. Except for one case (Case 25) in which the blood pressure was 175 systolic and 78 diastolic, no systolic tensions exceeded 170. All diastolic readings were essentially normal. Congestive cardiac failure in the absence of a cause other than amyloid involvement of the heart was thus encountered in 4 of the 29 cases. In this regard these cases resemble cases of primary systemic amyloidosis in which congestive cardiac failure is a common manifestation.[10]

Since the clinical features of an occasional case of cardiac amyloidosis may resemble those of constrictive pericarditis,[11] it is possible that pericardectomy might be undertaken in a case of amyloid disease wrongly diagnosed constrictive pericarditis. In these circumstances it might seem appropriate to establish by biopsy of the heart whether amyloid involvement is the basic difficulty. Amputation of the left auricular appendage might be a convenient method of performing such a biopsy. With this in mind, sections of the left auricular appendage from 11 of our 29 cases were studied in order to learn whether this procedure might be of aid in the diagnosis of cardiac amyloidosis in appropriate circumstances. In only 7 of the 11 cases, however, was amyloid found in the left auricular appendage.

COMMENT

The data presented suggest that amyloid deposition restricted largely or entirely to the heart is found principally among elderly patients, with a particular tendency for it to occur in men. The figures presented are consistent with the opinion of King in regard to the advanced age of patients with the condition, the average age in our 29 cases being 82 years. The average age was the same in the cases selected as to age as in those encountered in routine necropsy work. The average ages of patients with primary systemic amyloidosis [12] and with amyloid diseases complicating multiple myeloma [13] seem to be less than the average age of patients with amyloid deposition restricted to the heart.

10. Lindsay, S., and Knorp, W. F.: Primary Systemic Amyloidosis, Arch. Path. **39**:315 (May) 1945. Lindsay, S.: The Heart in Primary Systemic Amyloidosis, Am. Heart J. **32**:419 (Oct.) 1946; The Heart in Primary Systemic Amyloid Disease, Mod. Concepts Cardiovas. Dis. **17**:(N.P.) (June) 1948. Dahlin, D. C.: Amyloidosis, Proc. Staff Meet., Mayo Clin. **24**:637 (Dec. 21) 1949.

11. Findley, J. W., Jr., and Adams, W.: Primary Systemic Amyloidosis Simulating Constrictive Pericarditis, with Steatorrhea and Hyperesthesia, Arch. Int. Med. **81**:342 (March) 1948. Paul, O.: Chronic Constrictive Pericarditis, Mod. Concepts Cardiovas. Dis. **19**:83 (Nov.) 1950.

12. Parker, R. L.; Odel, H. M.; Logan, A. H., Jr.; Kelsey, J. R., Jr., and Edwards, J. E.: Primary Systemic Amyloidosis: Report of 2 Cases, M. Clin. North America **34**:1119 (July) 1950.

13. Bayrd, E. D., and Bennett, W. A.: Amyloidosis Complicating Myeloma, M. Clin. North America, **34**:1151 (July) 1950.

In reviewing the clinical features of our cases, we sought clues that might have led to a correct clinical diagnosis of amyloid deposition restricted to the heart. In none of the cases had a Congo red test for amyloid been done clinically. While it would be of interest to have data on the results of the test in this condition, it is doubtful that positive results would be obtained. This opinion is expressed in view of the fact that the total amount of amyloid in the body in cases in which amyloid deposition is restricted to the heart is much less than in the cases of disseminated amyloidosis, which is known to give positive results.

Amyloid disease of the heart may be suspected when an elderly patient has congestive cardiac failure without evidence of any other form of cardiac disease which might produce failure. The electrocardiograms of 10 of the patients in our series are reviewed in another communication.[14] From these, however, no specific diagnostic features can be derived.

SUMMARY AND CONCLUSIONS

The pathologic and clinical features of amyloid deposition restricted largely or entirely to the heart, as judged from 29 cases, are presented. The condition was observed only in elderly patients. In 19 of the 29 cases the condition was encountered in routine pathologic material; in only three of these cases was the patient less than 70 years of age. The remaining 10 of the 29 cases were found in a survey of the incidence of amyloid deposition restricted to the heart in 100 hearts from men and 100 hearts from women, each in the seventh, eighth, and ninth decades of life, the number of hearts totaling 600. No cases in this survey were discovered in the patients of the seventh decade. In the 200 hearts from men and women of the eighth decade 3 cases were discovered, all in men. From a like number of patients in the ninth decade of life, seven cases were encountered, five of which were in men and two in women.

The average age of patients with amyloid deposition restricted to the heart appears to be higher than that for patients with primary systemic amyloidosis or amyloid disease complicating multiple myeloma.

The condition, amyloid localized in the heart, may be associated with insignificant involvement of other organs. This is in contrast to primary systemic amyloidosis in which involvement of organs other than the heart may be extensive.

In 4 of the 29 cases of amyloid deposition restricted largely or entirely to the heart, congestive cardiac failure existed in the absence of other recognized cause of such failure.

Although there are no specific clinically diagnostic signs of amyloid deposition restricted to the heart, this entity should be suspected in the presence of congestive cardiac failure in an elderly patient unafflicted by hypertension, valvular or coronary arterial disease, or other conditions known to be associated with cardiac failure.

14. Josselson, A. J., and Pruitt, R. D.: The Electrocardiographic Findings in Cardiac Amyloidosis, unpublished data.

TUMORS PRODUCED BY METHYLCHOLANTHRENE IN THE DUCK

Papilloma, Squamous-Cell Carcinoma, and Hemangioma

R. H. RIGDON, M.D.
GALVESTON, TEXAS

THE DUCK has been used frequently for the experimental study of tumors. Fujinami and Hatano [1] in 1929 transferred a myxosarcoma of fowls to the duck. This experiment was repeated by others.[2] The duck has been used in the study of the Rous sarcoma.[3] This host has been used infrequently, however, for the production of tumors with chemical carcinogens. In 1945 Duran-Reynals and associates [4] stated that carcinogenesis in species of birds other than the chicken had not been reported. These investigators observed one tumor in a total of 52 ducks given intramuscular injections of methylcholanthrene dissolved in lard. This was a fibrosarcoma and was first observed nine months after the injection. In 1944 Zondek [5] repeatedly injected estradiol benzoate intramuscularly into white Pekin and mallard ducks but failed to observe the occurrence of any tumors after 42 weeks.

MATERIAL AND METHOD

Since the white Pekin duck has been used in this laboratory for many experimental studies during the past 10 years, it was considered advisable to use this bird for preliminary observations on carcinogenesis. In a concentration of 10 mg. per milliliter 20-methylcholanthrene in sesame oil was used for both subcutaneous and intramuscular injections. A 0.25% solution of methylcholanthrene in acetone was used for topical applications to the skin of the chest wall beneath the wing. Acetone was used as the control preparation on the skin of normal ducks. The total amount of methylcholanthrene injected into each duck and the frequency of the local applications of the carcinogen and acetone are given for the different experiments. The ducks used in these studies varied from 10 days of age to adults. All injections were made in the

From the Laboratory of Experimental Pathology, University of Texas School of Medicine.

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2. Gye, W. E.: A Note on the Propagation of Fujinami's Fowl Myxosarcoma in Ducks, Brit. J. Exper. Path. **12**:93-97, 1932. Purdy, W. J.: Propagation of the Fujinami Fowl-Myxosarcoma in Adult Ducks, Brit. J. Exper. Path. **13**:467-472, 1932.

3. Duran-Reynals, F.: Study of 3 New Duck Variants of the Rous Chicken Sarcoma, Cancer Res. **7**:99-102, 1947; Neoplastic Infection and Cancer, Am. J. Med. **8**:490-511, 1950.

4. Duran-Reynals, F.; Shrigley, E. W., and de Hostos, E.: Carcinogenesis Induced by Methylcholanthrene in Pigeons, Guinea Fowls, and Ducks, Cancer Res. **5**:11-17, 1945.

5. Zondek, cited by Hartwell, J. L.: Survey of Compounds Which Have Been Tested for Carcinogenic Activity, Publication No. 149, Ed. 2, Federal Security Agency, U. S. Public Health Service, 1951.

pectoral region on the right side. Originally the methylcholanthrene and/or acetone was carefully applied to the skin with a cotton applicator; however, this technique was discontinued after a short time, and the methylcholanthrene and acetone were dropped onto the skin from an eye dropper. The quantity used at any one time varied with the size of the bird; our aim was only to moisten the skin. When the methylcholanthrene was applied to the younger ducks, the down was removed. Some of the feathers were plucked from the older ducks during the time of treatment; however, no attempt was made to remove all the feathers at any time except in some ducks in which skin tumors developed after the discontinuation of the carcinogen.

The ducks with methylcholanthrene applied to the skin were kept in a battery in the laboratory. These birds were observed either at weekly intervals or more frequently during the time that tumors were present. When tumors were not present, the birds were placed in

TABLE 1.—*Neoplasms Observed in Ducks Following Subcutaneous and Intramuscular Injections of Methylcholanthrene*

Group	Total No.	Age, Days	Agent Injected	No. Injections	Total Amount, Ml.	Route	Neoplasms	Time of Killing, Days	
A *	8	14	MC † in liquid petrolatum	3	1.5	I. M.	1	D 115	40
								D 117	367
								D 125	549
								D 113	581
								D 114, 116	586
B	5	16	MC ‡ in sesame oil	1	0.5	S. C.	0	D 19	48
								D 16, 17, 18, 20	661
C	5	16	MC ‡ in sesame oil	1	0.2	S. C.	0	D 24	82
								D 21	273 §
								D 22, 23, 25	280
D	2	21	MC ‡ in sesame oil	1	1.0	I. M.	0	D 60	296
								D 67	642
E ‖	2	69	MC in liquid petrolatum	1	1.0	I. M.	0	D 90	582
F	2	77	MC in liquid petrolatum	1	3.0	S. C.	0	D 135	50
								D 136	306
G	2	82	MC in liquid petrolatum	3	3.0	I. M.	0	D 47	266 §
								D 43	550
H	3	82	MC in liquid petrolatum	3	3.0	S. C.	0	D 45	330
								D 42, 46	550
I	7	Adult	MC in liquid petrolatum	1	1.0	I. M.	1	D 30	407
								D 5, 7, 10, 21, 22	554
								D 9	592

* Two ducks, 118 and 119, normal and discarded after 582 days.
† 10 mg. of methylcholanthrene per milliliter of liquid petrolatum U. S. P.
‡ 8 mg. of methylcholanthrene per milliliter of sesame oil.
§ Duck died during experiment.
‖ Duck 86 had inflammatory mass removed on 579th experimental day.

an open duck pen in the laboratory yard and were examined at less frequent intervals. When a tumor developed, the duck was removed to the laboratory for more careful observations. All ducks were fed purina® "startena" and "growena" and were given water ad libitum.

The size of the subcutaneous and intramuscular injections varied from 0.2 to 3.0 ml. each. As shown in Table 1, some of the ducks were given 0.5 ml. of the carcinogen on each of three occasions less than a week apart. The maximum amount given to any duck during the experiment was 3 ml. (30 mg. methylcholanthrene).

A few of the birds died during the experiment, and usually autopsy was performed. Some were killed when moribund, and an autopsy was done. A majority were killed at various intervals and autopsy done immediately. Biopsy specimens were taken from the methylcholanthrene-treated skin, and some of the neoplasms were removed surgically. The length of each experiment is given in Tables 1 and 2. The longest interval during which any duck was observed after either a subcutaneous or an intramuscular injection was 661 days, and the longest period after the local application of methylcholanthrene to the skin was one year.

Sections were fixed in 4% solution of formaldehyde U. S. P. (formalin). Paraffin sections were stained routinely with hematoxylin and phloxine. Multiple step-sections were made on some of the blocks of skin and tumors.

EFFECT OF METHYLCHOLANTHRENE INJECTED SUBCUTANEOUSLY AND INTRAMUSCULARLY IN THE DUCK

Both the ducks receiving subcutaneous and those receiving intramuscular injections were observed closely for two to three weeks. Little local reaction occurred except in the two birds given 3 ml. subcutaneously, as shown in Group F (Table 1). On the 12th experimental day an indurated area measuring 2 by 3 cm. was observed at the site of injection in Duck 136. A biopsy specimen showed an acute and a chronic inflammatory reaction. No tumor tissue was present on the 366th day, when the bird was killed. The second bird, Duck 135, was killed on the 50th experimental day. There were a large amount of edema and necrosis and some hemorrhage at the site of injection in both the subcutaneous tissue and the adjacent muscle. No neoplastic tissue was present.

The first tumor found in the ducks given methylcholanthrene, either subcutaneously or intramuscularly, was observed in Duck 66, Group D (Table 1), on the 294th experimental day. Two days later this bird was killed. The tumor measured 8 by 8 cm. and was located on the right side medial to the thigh. There was no ulceration of the skin. The tumor was circumscribed and easily separated from the surrounding tissue. Only a thin margin of chronic inflammatory tissue surrounded an organizing blood clot. There were considerable necrosis, multiple giant cells, and numerous small blood vessels in the inflammatory process. No neoplastic tissue was found.

The second tumor was first observed in Duck 86, Group E (Table 1), on the 337th day following an intramuscular injection of 1 ml. of methylcholanthrene (10 mg.). The tumor measured 3 by 3 cm. It was firm in consistency and partially fixed to the surrounding tissue. It was located in the area of the injection. This tumor was observed for eight months, during which time there was no change in either the size or the gross appearance. On the 554th experimental day the tumor was removed and found to be only a mass of necrotic tissue surrounded by a capsule of inflammatory tissue.

Two birds, Ducks 5 and 113, Groups A and I (Table 1), given intramuscular injections of methylcholanthrene, were found to have a neoplasm in the pectoral muscle when killed on the 554th and 581st days, respectively (Fig. 1*A*). Grossly, these neoplasms were similar. No line of demarcation separated the tumor and the surrounding muscle. The tumor tissue was grayish-white and gelatinous in consistency. Histologically, there were areas in which the neoplasm was formed by small endothelium-lined sinuses, some of which extended between the muscle bundles. In some areas the tumor was composed of fat, fibrous tissue, mucoid material, and collections of lymphocytes (Fig. 1*B*, *C*, *D*, and *E*).

Of the 36 ducks shown in Table 1, 32, receiving either subcutaneous or intramuscular injections, had no reaction, or only chronic inflammation, at the site of injection of the methylcholanthrene. Two ducks appeared grossly normal on the 582d experimental day and were discarded. In only two, Ducks 5 and 113, Groups A and I (Table 1), did a neoplasm develop.

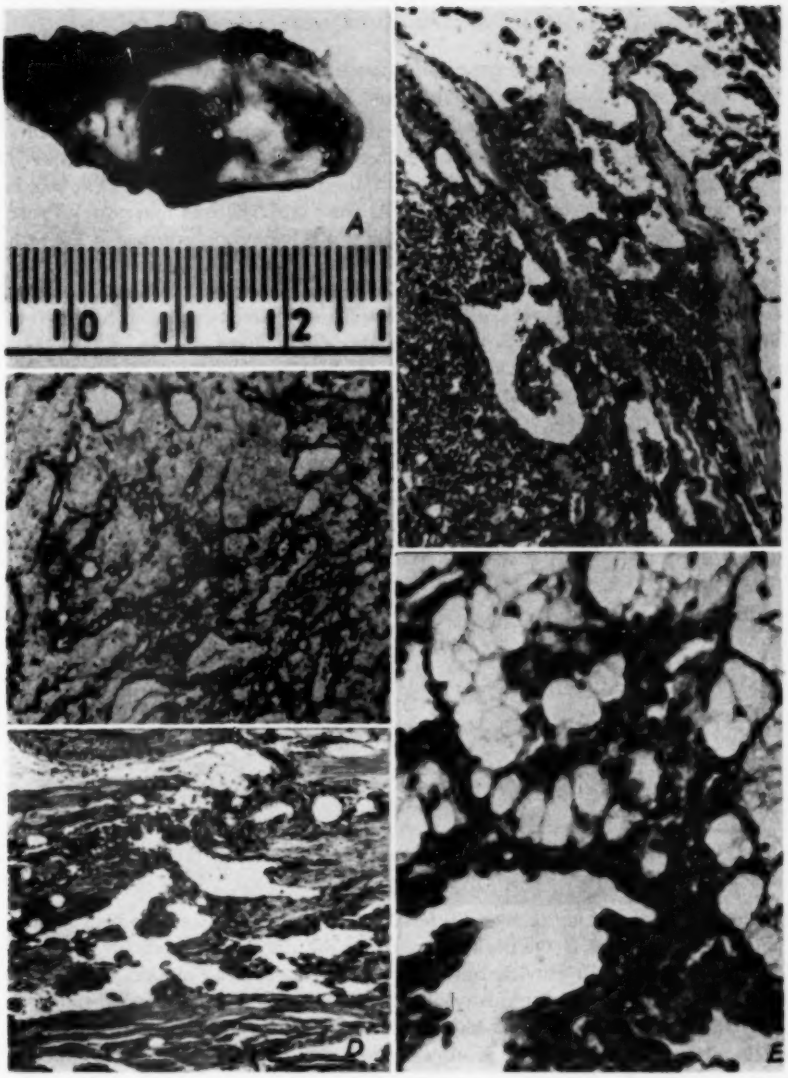

Fig. 1 (Duck 5).—(*A*) One milliliter of methylcholanthrene (10 mg.) in liquid petrolatum U. S. P. was injected intramuscularly 554 days before this tumor was removed.

(*B*) The neoplasm is vascular. Some of the sinuses are very large, while others are small. They are lined with endothelium-like cells. Hematoxylin and phloxine stain; × 96.

(*C*) Many large lipid cells infiltrate portions of this neoplasm. Hematoxylin and phloxine stain; × 96.

(*D*) Neoplastic tissue is present in the muscle. The lesion here is similar to that shown in *B*. Hematoxylin and phloxine stain; × 96.

(*E*) This shows the type of cell lining the sinuses and the large lipid cells present in the neoplasm. Hematoxylin and phloxine stain; × 420.

EFFECT OF METHYLCHOLANTHRENE APPLIED LOCALLY TO THE SKIN OF THE DUCK

Data on the ducks used in this experiment are presented in Table 2. The ages varied from 10 to 35 days when the experiment was begun. The methylcholanthrene in acetone was applied daily, with few exceptions, for 33 to 66 days. The maximum time during which any of these ducks were observed was one year. There was some variation in the degree of reaction to the methylcholanthrene in the skin of the different ducks. All the birds showed some edema and hyperemia after the carcinogen had been applied for two to three weeks. The most conspicuous macroscopic change was the desquamation of the epidermis, which continued to progress for two to three weeks after the treatments were discontinued. Many birds showed an excessive amount of desquamation, accompanied by a brownish, moist appear-

TABLE 2.—*Neoplasms Observed in Ducks Following Local Application of Methylcholanthrene to Skin*

Group	Total No.	Age, Days	Length of Treatment, Days	Agent Applied	No. of Ducks with Neoplasms			Time of Killing, Days	
					Papilloma	Squamous-Cell Carcinoma	Hemangioma		
A	5	33	66	MC *	4	1	1	D 137, 138, 141	366
								D 139	151 †
								D 140	33
B	5	13	48	MC	5	1	3	D 144	345
								D 145	283
								D 146	43
								D 147	249
								D 148	52
C	5	10	33	MC	4	2	4	D 240	138
								D 241, 242, 243, 244	182
D	5	10	33	Acetone	0	0	0	D 235, 239	182 ‡
								D 236	88
								D 237	90
								D 238	65
E	4	35	33	Acetone	0	0	0	D 185, 189	96
								D 188	40 †
								D 276	182 ‡

* A 0.25% solution of methylcholanthrene in acetone.
† Duck died.
‡ Duck discarded, grossly normal.

ance of the desquamating tissue. Bacterial infection did not appear to be a significant factor. In some of the ducks the feathers did not regrow normally after the topical application of methylcholanthrene. The treated areas of skin in some of these ducks had a light, brownish-yellow color for as long as six months.

A varying number of papillomata began to appear on the skin even before the application of the carcinogen was discontinued. The earliest papilloma was noted on the 42d day. Similar tumors continued to appear for three to four months, while the older papillomata disappeared spontaneously. Macroscopically, the base of some of these desquamating papillomata formed small hemorrhagic ulcers, which healed after several days. Usually these papillary tumors were 2 to 3 mm. in width at their base and 0.5 to 1.0 cm. in length (Fig. 2). Papillomata appeared on the skin of ducks in which the skin was treated with methylcholanthrene for periods longer than 33 days. Every papilloma either spontaneously disappeared or was removed for biopsy. None became macroscopically a malignant neoplasm and persisted as such.

Duck 140 was killed on the 33d day that methylcholanthrene was applied to the skin. Several histological sections were made from the treated area. The epidermis was several layers thick, with a definite differentiation of the cells into basal, granular, and squamous zones. Normally the dermis of the duck is only two to three cells thick. The hyperplastic epidermis had a moderate number of mitotic cells in the granular and basal layers. A varying amount of keratin covered the epidermis, and in some areas it was quite thick. In focal areas of the epidermis and on the overlying surfaces were collections of leucocytes, monocytes, and erythrocytes, many of which were undergoing degeneration. In many local areas the basal cells were atypically arranged, and frequently they extended down into the corium. Squamous cells with mitotic figures sometimes were present in areas of the corium.

Fig. 2 (Duck 145).—Methylcholanthrene was applied locally to the skin for 43 days, at which time these papillomata were photographed.

The latter infiltrations were consistent with the changes observed in a malignant neoplasm. These histological changes in the skin varied in the different ducks in relation to the time the carcinogen was applied and the sections were studied. In some ducks one or more histologically verified squamous-cell carcinomata were present. Squamous-cell carcinomata were demonstrated in the skin of four ducks treated with methylcholanthrene and acetone, as shown in Table 2.

Duck 240, Group C (Table 2), had methylcholanthrene in acetone applied to the skin for 33 days. On the 50th day a papilloma 3 mm. in diameter was observed. During the following 10 days this tumor gradually grew and was accidentally knocked off, leaving a superficial hemorrhagic base. A biopsy specimen was taken on the 63d experimental day. The lesion was a squamous-cell carcinoma (Fig. 3). There is no way of knowing the percentage of papillomata that may have shown

microscopic neoplastic changes. Papillomata and squamous-cell carcinomata continued to appear in some of the ducks as long as they lived. Duck 145 was killed on the 283d day of the experiment, and a squamous-cell carcinoma was present in the skin. No papillomata occurred in the skin of the ducks treated only with acetone and followed for either six months or one year. The down and feathers continued to grow. One, Duck 236, Group D (Table 2), was killed on the 88th day. The corium was edematous and showed a chronic inflammatory reaction. The epidermis was necrotic in some focal areas, but no areas were hyperplastic. There were no macroscopic or microscopic changes even to suggest a neoplasm in any of the nine acetone-treated ducks, Groups D and E (Table 2). Macroscopically, the skin was normal in all the acetone-treated ducks killed either six months or one year after treatment.

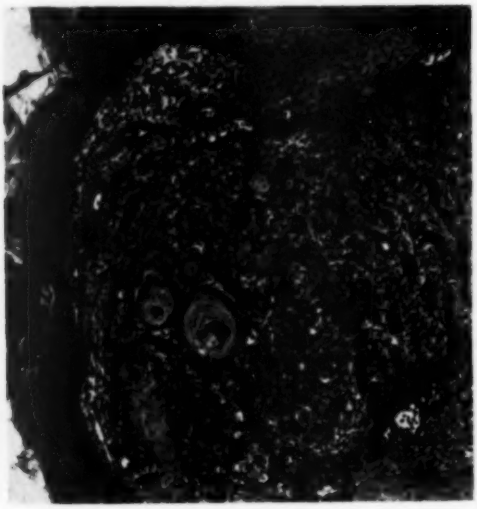

Fig. 3 (Duck 240).—Methylcholanthrene was applied locally to the skin for 33 days. Biopsy of a specimen taken on the 63d day from an ulcerated area representing the base of a desquamated papilloma showed a typical squamous-cell carcinoma. Hematoxylin and phloxine stain; × 96.

In some of the ducks which were treated locally with methylcholanthrene (Table 2) there developed one or more reddish-blue tumors in the subcutaneous tissue. The shortest interval between the first application of methylcholanthrene and the occurrence of one of these hemangiomatous tumors was 74 days. One or more of these neoplasms occurred grossly in 8 of 15 ducks treated with methylcholanthrene. Usually these tumors were less than 1 cm. in diameter. Frequently they produced a swelling on the surface of the skin; however, some appeared only as reddish-blue areas beneath the epidermis. The majority of these tumors grew slowly, and many completely disappeared. Others were removed surgically, or the ducks were killed and the lesion was studied histologically. The largest tumor occurred in Duck 145, Group B (Table 2). When removed surgically, on the 135th experi-

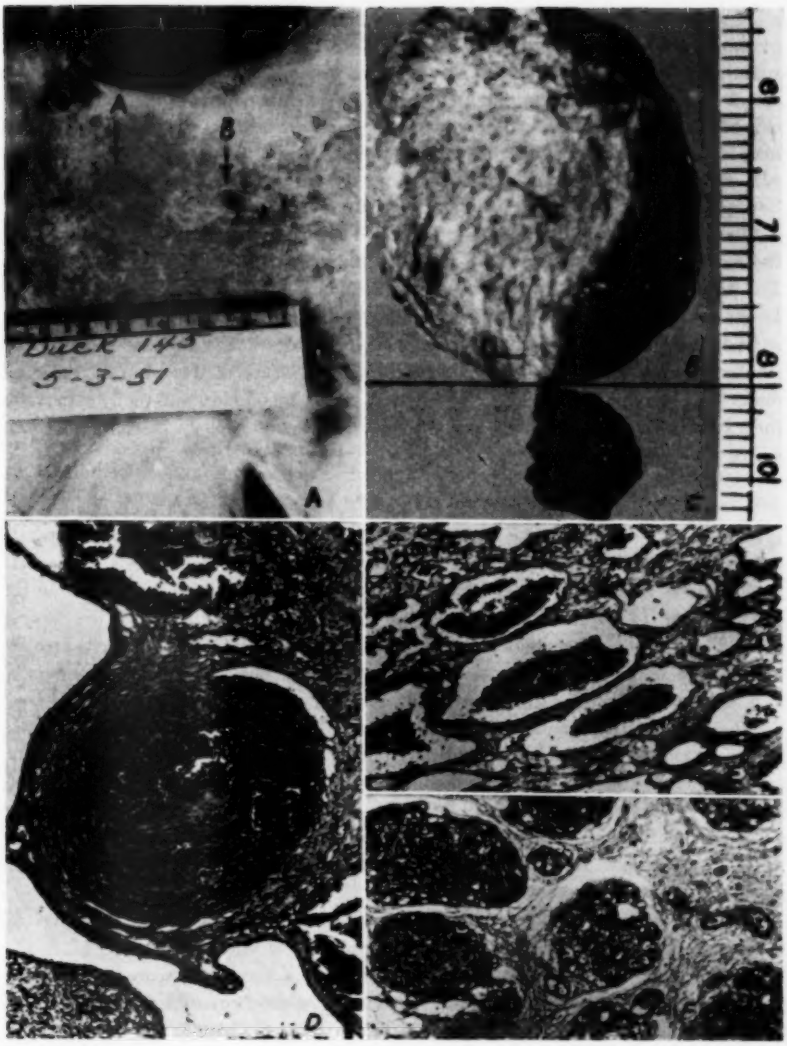

Fig. 4 (Duck 145).—(*A*) Methylcholanthrene was applied locally to the skin for 48 days. The two tumors shown at *A* and *B* were blue. They were removed on the 135th experimental day.

(*B* and *C*) Both tumors were grossly hemorrhagic.

(*D*) Some of the large sinuses (tumor *A*) are filled with thrombi. Hematoxylin and phloxine stain; × 90.

(*E*) The sinuses (tumor *A*) are lined with endothelial cells, and many are filled with red blood cells. Hematoxylin and phloxine stain; × 90.

(*F*) The sinuses (tumor *B*) are small. They are lined with endothelial cells, and many are filled with red blood cells. Hematoxylin and phloxine stain; × 90.

mental day, the tumor measured 3 by 2.5 by 1.5 cm. A second, smaller, tumor of similar appearance was removed at the same time (Fig. 4*A*, *B*, and *C*). Both tumors were demarcated from the adjacent tissue. There apparently was no recurrence of the tumors, although several other hemangiomata did develop in the skin of this bird after removal of the two neoplasms. The tumors were characterized histologically by innumerable blood sinuses lined with endothelium-like cells (Fig. 4*D*, *E*, and *F*). Some of the larger sinuses were occluded by recent thrombi. Groups of lymphocytes were present about the neoplastic tissue.

In one of the ducks (242), Group C (Table 2), a blue tumor developed on the margin of the wing in the area where some large feathers had been plucked. The tumor measured 2 by 1.5 cm. when removed on the 65th experimental day. In the center was a blood clot 1 cm. in diameter. A chronic granulomatous reaction surrounded the blood clot. There was no evidence of a neoplastic change. A similar, but smaller, lesion occurred on the outer surface of the wing of the same duck. The center of the lesion degenerated, and a blood clot was easily removed; the cavity refilled with blood, and this clot was spontaneously discharged. The entire lesion gradually became fibrotic.

COMMENT

This study on the white Pekin duck shows that papillomata, squamous-cell carcinomata and hemangiomata develop after multiple local applications of methylcholanthrene in acetone to the skin. Papillomata and squamous-cell carcinomata usually begin to appear approximately 40 days after the beginning of the topical applications, and the hemangiomata, about a month later. Of the 15 ducks treated locally, one or more papillomata were found in 13; one or more hemangiomata in 8, and squamous-cell carcinomata in 4. It would seem that a regression occurred histologically in the squamous-cell carcinomata, but it is difficult to establish this point. It can be said, however, that only one of the ducks had a lesion that would even suggest a squamous-cell carcinoma histologically when killed 6 to 12 months after the topical application of methylcholanthrene. Friedewald and Rous [6] have reported the regression of experimental tumors produced by methylcholanthrene in the rabbit, and Rous and Kidd [7] observed a similar regression of tumors produced by tar in the rabbit.

White Pekin ducks, after the subcutaneous or the intramuscular injection of varying amounts of methylcholanthrene in sesame oil or liquid petrolatum U. S. P., were observed for 18 months or longer. In two of the ducks a malignant neoplasm developed in the pectoral muscle that histologically resembled a blood vessel tumor. The two tumors were found at autopsy approximately 18 months after the injection of the carcinogen. There were no metastases.

The local occurrence of inflammatory masses following the subcutaneous injection of methylcholanthrene makes it necessary to confirm all suspected lesions by

6. Friedewald, W. F., and Rous, P.: Initiating and Promoting Elements in Tumor Production: Analysis of Effects of Tar, Benzpyrene, and Methylcholanthrene on Rabbit Skin, J. Exper. Med. **80**:101-126, 1944.

7. Rous, P., and Kidd, J. G.: Conditional Neoplasms and Subthreshold Neoplastic States: Study of Tar Tumors of Rabbits, J. Exper. Med. **73**:365-390, 1941.

histological examination. No tumors have been found in the ducks except in the areas where methylcholanthrene was applied locally or injected subcutaneously or intramuscularly.

There are many similarities in the features of neoplasms in the duck to those previously described by others in the production of tumors in the rabbit and the mouse.[8] It is of interest to note the frequent occurrence of hemangiomatous neoplasms in the duck following the topical application of methylcholanthrene and the occurrence of a similar tumor in the muscle following an intramuscular injection of methylcholanthrene. The gross and histological features of the latter tumor would suggest that it is a malignant neoplasm. Hemangioendotheliomata in mice following the use of carcinogens has been reported by several investigators.[9]

The relation of trauma to the development of squamous-cell carcinomata and hemangiomata in the duck may be significant. The trauma results from the plucking of feathers. This factor is being studied now.

SUMMARY

Papilloma, squamous-cell carcinoma, and hemangioma occurred in white Pekin ducks after the topical application of methylcholanthrene to the skin. A hemangiomatous tumor was also observed in the pectoral muscle after the intramuscular injection of the same carcinogen. Histological changes occurred in the skin of the duck treated with methylcholanthrene similar to those described by other investigators in the rabbit and the mouse. Apparently, all the papillomata, the squamous-cell carcinomata, and the hemangiomata regressed. None of the neoplastic lesions were observed to metastasize. The first papillomata appeared approximately one month, and the first hemangiomata approximately two months, after the first application of methylcholanthrene to the skin. The neoplasm occurring in the muscle was first observed approximately 18 months after the intramuscular injection of methylcholanthrene. In these preliminary experiments some information has been gained relative to the amount of the carcinogenic agent necessary to be injected intramuscularly or applied locally to the skin for the development of tumors. The duck is a very satisfactory host for the experimental study of neoplasms produced by methylcholanthrene.

8. Cramer, W., and Stowell, R. E.: Early Stages of Carcinogenesis by 20-Methylcholanthrene in the Skin of the Mouse: Microscopic Tissue Changes, J. Nat. Cancer Inst. **2**:379-402, 1941-1942. Friedewald and Rous.[6]

9. Andervont, H. B.: Induction of Hemangio-Endotheliomas and Sarcomas in Mice with O-Aminoazotoluene, J. Nat. Cancer Inst. **10**:927-941, 1950. Hartwell, J. L., and Stewart, H. L.: Action of 5, 9, 10-Trimethyl-1, 2-Benzanthracene on Skin of Mouse, ibid. **3**:277-285, 1942. Steiner, P. E.: Comparative Pathology of Induced Tumors of Salivary Glands, Arch. Path. **34**:613-624, 1942.

DEGENERATIVE CHANGES IN LIVER ASSOCIATED WITH ASPIRATION OF VERNIX AND HYALINE MEMBRANE FORMATION IN LUNGS IN INTRAUTERINE ANOXIA

MAJOR ROBERTO E. BENITEZ
MEDICAL CORPS, UNITED STATES ARMY

PRIMARY intrauterine anoxia has been increasingly recognized in recent years as a cause of many stillbirths and many deaths in the newborn. It is probably responsible for the greatest number of infant deaths in the neonatal period. The morphological changes which characterize this condition have been described in great detail in the literature.[1] The lung findings most commonly described at autopsy have been atelectasis, aspirated vernix caseosa, and pneumonia.

In recent years, the finding of a hyaline-like membrane coating alveoli, alveolar ducts, and bronchioles has been increasingly reported in autopsy studies. The exact origin of the membrane, as well as its role in the mechanism of death, has not yet been elucidated to the complete satisfaction of most pathologists, obstetricians, and pediatricians. Thus, while some authors [2] definitely consider a hyaline membrane as the most important cause of death in newborn infants, others still sign these cases out with the old diagnoses of "stillbirth, due to unknown cause," "prematurity," "neonatal death," "fetal atelectasis," or other diagnosis.

The effects of anoxia on the liver have been recorded by many workers.[3] In a recent report by Gruenwald [4] on a study of 200 autopsies of infants who died from intrauterine anoxia, the liver was found to exhibit various alterations, ranging from cloudy swelling to foci of necrosis. A prominent and frequent finding in this study was fatty metamorphosis of the liver. Still, there are those who regard fatty metamorphosis of the liver of newborn infants with skepticism. Recently, Dorkin and Weinberg [5] contended that the presence of histologically demonstrable fat in the liver

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1. (*a*) Clifford, S. H.: J. Pediat. **18**:567, 1941. (*b*) Potter, E. L.: Pathology of the Fetus and the Newborn, Chicago, Year Book Publishers, Inc., 1952. (*c*) Potter, E. L., and Adair, F. L.: Fetal and Neonatal Death: A Survey of the Incidence, Etiology, and Anatomic Manifestations of the Conditions Producing Death of the Fetus in Utero and the Infant in the Early Days of Life, Chicago, University of Chicago Press, 1949.

2. (*a*) Miller, H. C., and Hamilton, T. R.: Pediatrics **3**:735, 1949. (*b*) Miller, H. C., and Jennison, M. H.: Pediatrics **5**:7, 1950. (*c*) Potter.[1c]

3. (*a*) Boyd, W.: A Text-Book of Pathology: An Introduction to Medicine, Ed. 5, Philadelphia, Lea & Febiger, 1947. (*b*) Clifford.[1a] (*c*) Gillman, J., and Gillman, T.: South African J. M. Sc. **13**:11, 1948. (*d*) Trowell, O. A.: Correspondence, Nature, London (Supp.) **151**:730, 1943.

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5. Dorkin, J. R., and Weinberg, T.: Arch. Path. **48**:578, 1949.

Data on Forty-Four Infants with Pathological Alterations in Lungs and/or Liver

Case No.	Intrauterine Age	Period of Survival	Condition Leading to I. U. Anoxia	Body Weight, Gm.	Microscopic Examination of Lungs	Aspirated Vernix	Hyaline Membrane	Fatty Liver
7	6½ mo.	11 hr.	Undetermined	1,180	Partial expansion; postnatal atelectasis	Yes	Yes	++
8	6½ mo.	24 hr. 40 min.	Undetermined	1,200	Partial expansion; postnatal atelectasis	Yes	Yes	....
11	6½ mo.	8 hr.	Placenta previa	1,640	Unexpanded	Yes	No	No
19	6½ mo.	24 hr.	Prem. sep. placenta	860	Overdistention; postnatal atelectasis	Yes	No	++
29	5½ mo.	Stillborn	Undetermined	654	Unexpanded	Yes	No	No
31	6½ mo.	10½ hr.	Placenta previa	465	Partial expansion	Yes	Yes	++
34	6½ mo.	Stillborn	Placenta previa	1,225	Unexpanded	No	No	+
36	6½ mo.	Stillborn	Prem. rupt. membranes	900	Unexpanded.	Yes	No	+
38	7 mo.	12 hr.	Undetermined	1,600	Partial expansion	Yes	No	No
41	6 mo.	1 hr.	Long labor	684	Partial expansion	Yes	No	+
42	7 mo.	2 days	Undetermined	1,730	Partial expansion	Yes	Yes	++++
46	8½ mo.	15 hr.	Undetermined	1,560	Partial expansion; bronchopneumonia	Yes	No	+++
48	6 mo.	3 hr. 10 min.	Prem. rupt. membranes	1,250	Unexpanded	Yes	No	No
49	7 mo.	1 hr.	Undetermined	850	Partial expansion	Yes	Yes	++++
52	7 mo.	4 days	Undetermined	909	Partial expansion; bronchopneumonia	Yes	No	No
53	6 mo.	15 hr.	Placenta previa	1,297	Partial expansion	Yes	Yes	....
57	6½ mo.	68 hr.	Undetermined	778	Bronchopneumonia	No	No	+++
64	6½ mo.	1 hr.	Infarcts of placenta	895	Unexpanded	Yes	No	++
66	8 mo.	8½ hr.	Shock	1,700	Expanded	Yes	Yes	++
67	5½ mo.	Stillborn	Undetermined	520	Unexpanded	Yes	No	No
69	6 mo.	7 hr.	Placenta previa; prem. separation	1,230	Partial expansion	Yes	Yes	....
70	5½ mo.	10 min.	Undetermined	440	Unexpanded	Yes	No	++
71	5½ mo.	1 hr. 45 min.	Undetermined	478	Unexpanded	Yes	No	No
73	8 mo.	10 hr. 15 min.	Eclampsia	1,540	Partial expansion	Yes	Yes	++
77	7½ mo.	33 hr.	Prem. sep. placenta	801	Atelectasis; bronchopneumonia	Yes	No	++
81	8½ mo.	67 hr.	Undetermined	1,975	Partial expansion; postnatal atelectasis	Yes	No	+++
92	6½ mo.	13 hr.	Prem. rupt. membranes	755	Undeveloped alveoli	Yes	No	No
96	7 mo.	10 days	Undetermined	775	Partial expansion; postnatal atelectasis	Yes	No	No
3	8½ mo.	40 min.	Undetermined	2,067	Unexpanded; congestion	Yes	No	+
4	Term	Stillborn	Dystocia	3,135	Unexpanded; congestion; phagocytosis	Yes	No	No
5	Term	Stillborn	Dystocia	3,010	Overdistended alveoli	No	No	+
10	8½ mo.	Stillborn	Cord around neck Preeclampsia	2,800	Unexpanded	Yes	No	....
30	5 mo.	9½ hr.	Undetermined	2,850	Partial expansion	Yes	No	+++
33	Term	Stillborn	Undetermined	3,327	Unexpanded	Yes	No	+
35	Term	Stillborn	Undetermined	2,700	Expanded	Yes	No	++
39	7 mo.	Stillborn	Prem. sep. placenta	2,412	Unexpanded	Yes	No	++++
40	Term	2 hr.	Infarct of placenta Pulseless cord	2,380	Partial expansion	Yes	No	++++
51	8½ mo.	39 hr.	Undetermined	2,270	Partial expansion	Yes	Yes	++++
74	Term	8 days	Undetermined	2,500	Patchy atelectasis; interstitial pneumonia	No	No	+++
76	Term	Stillborn	Undetermined	2,930	Unexpanded	Yes	No	++++
79	Term	Stillborn	Undetermined	3,100	Unexpanded	Yes	No	No
83	Term	Stillborn	Undetermined	3,060	Unexpanded	Yes	No	++
91	9½ mo.	2 days	Undetermined	2,130	Bronchopneumonia	Yes	Yes	++
94	9 mo.	15 hr. 45 min.	Prem. sep. placenta Prolapse of cord	2,440	Expanded; hemorrhages	Yes	Yes	+

of a newborn is physiological. Their study of 31 autopsies on infants less than 14 days of age is the first published attempt to correlate birth weight with the presence of fat in the liver. Dorkin and Weinberg concluded that hepatic fat storage is probably a preparation for birth.

It is the purpose of this study to analyze the findings in the lungs and liver in a series of 100 autopsies performed on newborn and stillborn infants and to determine whether fatty metamorphosis of the liver could be interpreted as a pathological phenomenon related to intrauterine anoxia, with particular consideration to its relationship to birthweight.

MATERIAL AND METHODS

One hundred autopsies performed at Walter Reed Army Hospital during the years 1937 to 1946 on stillborn and newborn infants who died before the age of 11 days were studied. The cases selected were those having a final pathologic diagnosis of "stillbirth," "prematurity," "fetal atelectasis," "intrauterine anoxia," or "neonatal death." Those cases in which death was due to cerebral hemorrhage, trauma, malformations, syphilis, or erythroblastosis were not included.

The clinical histories and gross autopsy findings in these cases were reviewed. The formalin-fixed, hematoxylin and eosin stained sections in each case were studied and the data recorded and tabulated (Table). In most of the cases there were two or more lung sections and one or more of liver. It was not possible to determine from which lobe the liver sections had been taken. In cases in which the hematoxylin and eosin sections showed vacuolation of the hepatic cells, fat stains were done on frozen sections of the liver to determine whether this was fatty metamorphosis or fat-free vacuolation. The sections were stained by the supersaturated isopropanol method with use of Oil Red O dye. The amount of fat present was graded from 0 to 4+ as follows:

0 —an occasional stained droplet or none.

1+ —minimal: occasional stained droplet in most of the lobules.

2+ —moderate: stained droplets in several liver cells in each lobule.

3+ —marked: stained droplets in approximately half of the cells in each lobule.

4+ —very marked: almost all cells contained stained droplets.

RESULTS

Cases which lacked essential maternal clinical data and/or gross and histologic examination of the liver and lungs, as well as those showing autolysis of the tissues, were eliminated. Thus, a total of 57 cases were considered adequate for study. Although specific mention of fetal distress before or during labor was lacking in many histories, in all liveborn infants there was respiratory distress at or shortly after birth. In all of these this marked respiratory distress led directly to death. Methods of resuscitation, particularly the use and concentration of oxygen, could not be evaluated for lack of details.

Conditions Leading to Intrauterine Anoxia.—In 29 cases it was possible to incriminate one or more complications of pregnancy or labor as possible causes of intrauterine anoxia and fetal distress: first, the condition most commonly encountered, premature separation of the placenta (nine cases); second, placenta previa (six cases); and third, early rupture of the membranes (five cases). The others included high fever, dystocia, preeclampsia, eclampsia, infarct of placenta, cord accident, long labor, unexplained hemorrhage, and shock.

Bronchopneumonia.—Evidence of bronchopneumonia was seen in eight cases. In some there was a diffuse interstitial pneumonia; in others a patchy, often coalescing, bronchopneumonia developed. The earliest occurrence was in Case 23, that of an infant who died 23 minutes after birth. Bronchopneumonia was not found in stillborn.

Atelectasis.—This finding was common to all. Two types could be recognized: One type was the usual so-called fetal atelectasis with non-air-bearing, partly separated, alveolar walls. It has been demonstrated[6] that the small irregular spaces between the alveolar walls contain amniotic fluid. This cannot be appreciated in routine sections after dehydration and embedding. The other type was postnatal or resorption atelectasis in which air, once present in the alveoli, was resorbed, leaving

Fig. 1.—Section of a lung showing a respiratory bronchiole, in one portion of which a hyaline membrane covers the denuded wall. The epithelium in the other portion is intact but is separated from the wall, owing to shrinkage of the tissue. Note the severe resorption atelectasis. Hematoxylin and eosin; × 100.

large areas of collapsed alveoli with the walls in close apposition. The resorption type of atelectasis was invariably seen in association with hyaline membranes (Fig. 1).

Aspiration of Vernix Caseosa.—Evidence of aspiration of vernix caseosa was found in 40 infants. It was most commonly seen as masses of pink-staining material in the alveoli or bronchioles, sometimes forming irregular homogeneous masses or having a fine fibrillar structure. Occasionally, epithelial cells could be identified in this material (Fig. 2). These cells usually were flattened, and of spindle or oval shape, with eosinophilic cytoplasm and round vesicular nuclei. In several instances

6. Davis, M. E., and Potter, E. L.: J. A. M. A. **131**:1194, 1946.

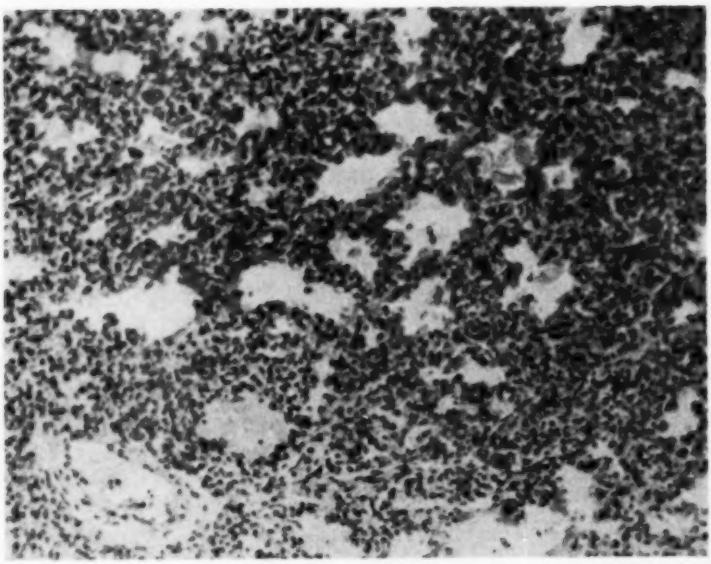

Fig. 2.—Section from a lung of a premature infant (Case 31). Note the vernix filling one alveolus and early membrane formation. Many epithelial cells are also seen in the alveoli. Hematoxylin and eosin; × 100.

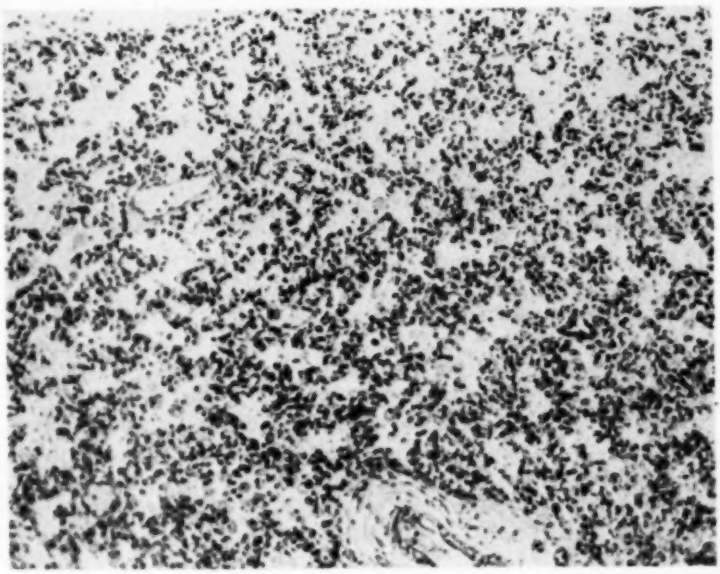

Fig. 3.—Section from a lung of a stillborn infant showing phagocytes in the alveoli and aspirated vernix. Hematoxylin and eosin; × 100.

there was active phagocytosis of the aspirated vernix (Fig. 3). One such instance occurred in a stillborn infant. The amount of vernix found varied widely. The lungs were not necessarily aerated, but, on the contrary, in many instances it was possible to identify vernix in unexpanded alveoli. Three infants had associated bronchopneumonia.

Of these 40 infants who had evidence of aspiration of vernix, 25 showed fatty metamorphosis in the liver. In 11, the liver showed no fat.

Hyaline-like Membrane.—A definite hyaline-like membrane in many alveoli, alveolar ducts, and respiratory bronchioles was seen in 12 infants. In all of these there was evidence of aspirated vernix (Fig. 4). All were premature infants; none were stillborn. Most membranes were found in infants weighing from 1,000 to 2,440

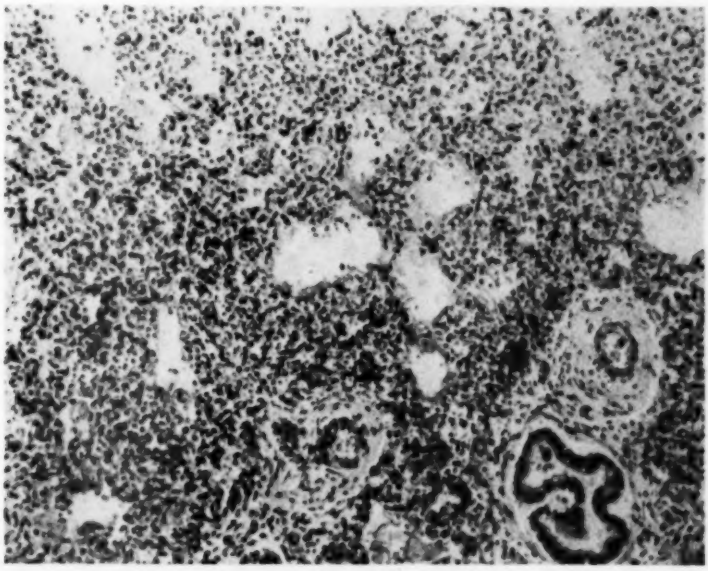

Fig. 4.—Section of a lung of a premature infant. A definite hyaline-like membrane is seen in several alveoli and alveolar ducts. Other alveoli contain aspirated vernix. Note also the resorption atelectasis. Hematoxylin and eosin; × 100.

gm. One weighed 850 gm. In one the alveoli were unexpanded. In the others there were partial to almost complete expansion of the alveoli and large areas of resorption atelectasis. In some the lungs showed overdistended alveoli, a finding usually associated with artificial respiration. There was bronchopneumonia in one infant. The earliest evidence of a membrane was seen in an infant who died one hour after birth. In all cases, except three that had no sections of the liver, there was fatty metamorphosis.

Degenerative Changes in the Liver.—In studying the sections of liver for evidence of degeneration, such changes as cloudy swelling, occasional foci of necrosis, and slight lymphocytic infiltration were occasionally noted. However, they were not

consistent enough to be convincing as positive evidence of degeneration. On the other hand, the presence of vacuoles in the liver cells was a frequent finding and was considered more significant (Fig. 5). With two exceptions, all sections of liver showing vacuolation in the hematoxylin and eosin stained preparation exhibited fatty metamorphosis by the fat stain. In the positive cases, occasional cells containing unstained vacuoles could be seen among the brightly stained neighboring cells of the lobule.

Since Dorkin and Weinberg [5] found that infants weighing less than 2,000 gm. had 0 to 1+ fat in the liver and those weighing over 2,000 gm. had an increasing amount of fat, our cases were separated into two groups according to body weight.

Of the 16 cases in which the weight of the infant was over 2,000 gm., there was visible fat in the liver of 13. Four had 1+, three had 2+, two had 3+, and four

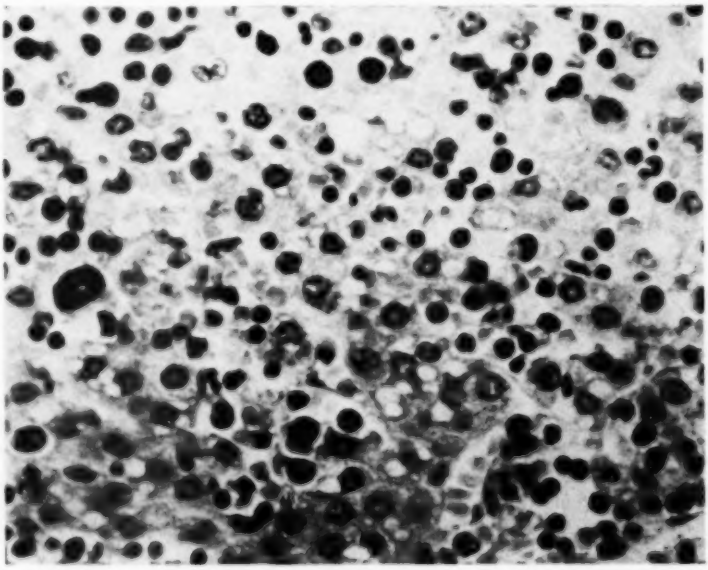

Fig. 5.—Portion of a field from a liver showing marked (3+) vacuolation. The majority of these vacuoles stain positively with special stain. In two cases there was fat-free vacuolation. Hematoxylin and eosin; × 550.

had 4+. In two full-term infants there was no fat. In one there was no section of the liver. All but two infants had aspirated vernix. Only three, all premature, had hyaline membranes. In the group of 28 infants weighing less than 2,000 gm., there was fatty metamorphosis of the liver in 16. Three had 1+ fat. The others showed more marked alteration, one up to 4+. Fourteen of these infants had evidence of aspirated vernix; six had hyaline membranes, and one had bronchopneumonia.

In the two groups, a total of 29 infants had evidence of fatty liver. Of these, 25 had evidence of aspirated vernix in the lungs. Of this number, nine also had evidence of a hyaline membrane. Of the remaining four, two had evidence of bronchopneumonia and two had clinical evidence of intrauterine anoxia.

COMMENT

It is generally accepted that the human fetus normally exhibits some degree of intrauterine respiration. When the oxygen supply to the fetus is inadequate, it is stimulated to increased respiratory efforts. The activity of the anoxic fetus results in the freeing of more cells and vernix from the surface of the body than is normal. This is aspirated by the fetus, together with the amniotic fluid. Potter [1b] states that the constant absorption of fluid permits concentration of the solid material, and the alveolar ducts may become distended by epithelial cells and vernix caseosa.

The finding of aspirated vernix caseosa in infants with a hyaline membrane would tend to support the theory [7] that the membrane develops from the aspirated vernix after contact with air under tension. According to this theory, the material then becomes intimately attached to the alveolar wall. The resulting membrane, or coat, produces respiratory embarrassment by interfering with the exchange of gases in the alveolus. The obstruction of respiratory bronchioles and larger air passages results in the resorption atelectasis which is invariably found in these cases, a complication that causes further respiratory difficulty and death of the infant.

Although in our study vernix was noted in the patients with a membrane, in the majority of instances of aspiration of vernix a membrane was not formed. In Case 96, for example, in an infant who survived 10 days the lungs showed evidence of aspirated vernix at autopsy yet no membrane developed. In this case, however, the amount of aspirated vernix was slight.

This theory of the pathogenesis of the hyaline membrane has been seriously questioned. Some workers [8] have proposed that the membrane represents a reaction to injury of pulmonary epithelium. Evidence for this lies in the fact that similar membranes have been described in the lungs of patients with influenza, Streptococcus pneumonia, radiation pneumonitis, uremia, and rheumatic pneumonia and in the lungs of those suffering from the inhalation of noxious gases. Brannan and Goodpasture [9] suggested that the membrane is formed from fibrin in the alveoli in influenza as a result of damage to the capillary bed. Bruns and Shields [10] advanced the theory that high-concentration oxygen therapy is injurious to alveolar capillaries and may be a factor in the development of a hyaline membrane in infants.

In our study, a membrane was found in one infant who died shortly after birth, ruling out the possibility of oxygen therapy as the causative agent in this particular case. Similar cases, however, have been found by others.[1b] Although no membranes have been seen in stillborn infants, a few cases are mentioned in the literature [2a] in which masses of hyaline-like material were found in the lungs of stillborn infants. Furthermore, membranes have been found in infants who had no oxygen, as well as in others for whom the oxygen concentration used was in the safe range.

7. Farber, S., and Sweet, L. K.: Am. J. Dis. Child. **42**:1372, 1931. Farber, S., and Wilson, J. L.: Arch. Path. **14**:437, 450, 1932. Opie, E. L.; Blake, F. G.; Small, J. C., and Rivers, T. M.: Epidemic Respiratory Disease: The Pneumonias and Other Infections of the Respiratory Tract Accompanying Influenza and Measles, St. Louis, C. V. Mosby Company, 1921.

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9. Brannan, D., and Goodpasture, E. W.: Arch. Int. Med. **34**:739, 1924.

10. Bruns, P. D., and Shields, L. V.: Am. J. Obst. & Gynec. **61**:953, 1951.

A simple explanation of the effects of anoxia in tissues [10] exists in the fact that in intrauterine anoxia there is interference with cell metabolism, resulting in increased lactic acid with decreased pH of the blood. This causes damage to blood vessels, with loss of tone, dilatation, and escape of plasma and red blood cells, and is manifested by petechial hemorrhages in many organs and edema of the tissues. This concept can be applied to explain the origin of the hyaline membrane. The damage to the alveolar capillaries results in exudation of plasma into the alveoli, with deposition of fibrin. All attempts to demonstrate fibrin by special methods have been futile. It is significant that Wolbach [11] stated some time ago that the altered fibrin in a diphtheritic membrane failed to stain. More recently Klemperer [12] observed that certain forms of "fibrinoid" do not stain in the usual manner, probably because of differences in chemical composition. If this concept is correct, it is easy to understand why similar pathological changes are found in such widely divergent conditions as infection, anoxia, hyperergy, chemical irritation, and radiation injury. The fact that some membranes in stillborn and newborn infants contain fat, as demonstrated by special stains, may be an indication merely that some aspirated vernix is mixed with it. This, then, can be considered a coincidental finding peculiar to this very special group of cases.

Dorkin and Weinberg [5] postulated that "fatty liver" was a normal finding in near-term infants. Their observations indicate that deposition of fat increases noticeably when the fetus attains a weight of 2,000 gm. They explained this phenomenon as a preparation of the fetus for birth. In support of this theory, they referred to previous experimental studies. Boyd and Wilson [13] showed that the blood in the umbilical vein of humans contains higher concentrations of certain lipid materials, mainly phospholipids and cholesterol, than does the blood in the umbilical arteries. Imrie and Graham [14] found that fat accumulates in the liver of guinea pigs during the latter part of gestation. Needham,[15] studying the respiratory quotient in the human infant, demonstrated that in utero the quotient is 1.0, indicating that carbohydrate is almost the only source of energy. Benedict and Talbot [16] found the respiratory quotient after birth to be approximately 0.90, indicating glycogen utilization. This, however, is soon depleted and by the third day the value reaches 0.73, indicating utilization of fat almost exclusively. Later, as the infant is able to utilize the carbohydate in the milk feedings, the respiratory quotient again increases.

In the light of this experimental evidence, Dorkin's and Weinberg's [5] findings of "fatty livers" in full-term and near-term infants is quite significant. The problem could be elucidated by devising an experiment, possibly by using fatty acids with "tagged carbons," to determine conclusively that the absorbed phospholipids are stored as neutral fat in the liver, as well as to determine the exact amount deposited.

11. Wolbach, S. B.: Bull. Johns Hopkins Hosp. **30**:104, 1919.

12. Klemperer, P.: In Seminar on Arterial Pathology, Johns Hopkins Hospital, Baltimore, Maryland, May 6, 1952.

13. Boyd, E. M., and Wilson, K. M.: J. Clin. Invest. **14**:7, 1935.

14. Imrie, C. G., and Graham, S. G.: J. Biol. Chem. **44**:243, 1920.

15. Needham, J.: Chemical Embryology, New York, The Macmillan Company, 1931, Vol. 2.

16. Benedict, F. G., and Talbot, F. B.: The Physiology of the New-Born Infant: Character and Amount of the Katabolism, No. 233, Carnegie Institution of Washington, 1915.

Dorkin and Weinberg [5] found no definite correlation with the associated pathologic changes. There was no mention in their work of clinical or morphological evidence of intrauterine anoxia. On the basis of their conclusions, we should have found at least 2+ fatty metamorphosis in all infants weighing over 2,000 gm. in this series. This, however, was not the case. Moreover, our observations indicate that in most instances of fatty metamorphosis of the liver, there was clinical or morphological evidence in intrauterine anoxia.

Aterman [17] recently reported finding glycogen-free, fat-free vacuoles in the regenerating livers of rats as early as five minutes after partial hepatectomy. These vacuoles are probably glycoproteins or lipoproteins. There are several other reports [18] of similar fat-free vacuoles in the liver in anoxic states. In our study we found two cases in which fat-free vacuolation could be observed. In addition, in most of the cases of fatty metamorphosis, occasional fat-free vacuoles could be demonstrated.

SUMMARY AND CONCLUSIONS

Observations from 57 autopsies on stillborn and newborn infants are presented.

An attempt has been made to correlate degenerative changes in the liver with intrauterine anoxia and the finding of aspirated vernix caseosa and a hyaline-like membrane in the lungs.

A few of the theories of the etiology of the hyaline-like membrane are discussed.

Our results suggest that fatty metamorphosis of the liver of premature infants is a pathological finding and is probably related to intrauterine anoxia.

Further experimental work is necessary to prove conclusively that the presence of histologically demonstrable fat in the liver of full-term and near-term infants is physiological.

17. Aterman, K.: A. M. A. Arch. Path. **53**:197, 1952.
18. Trowell.[3d] Price, J. M., and Laird, A. K.: Cancer Res. **10**:650, 1950.

MALAKOPLAKIA OF URINARY BLADDER

Report of Four Cases and Discussion of Etiology

VIRGIL R. BLEISCH, M.D.
AND
NADYA F. KONIKOV, M.D.
BOSTON

MALAKOPLAKIA of the urinary bladder, first diagnosed by von Hansemann in 1903, has become a well-defined gross pathologic entity.[1] The disease is characterized by soft plaques which have given the lesion its name. These plaques are yellow to brown in color and often have central umbilication or ulceration and peripheral hyperemia. Microscopically, they are formed predominantly of large mononuclear phagocytic cells, some of which contain calcospherites of Michaelis and Gutmann.[2] Reported cases of the lesion are rare, a total of 59 now being available, in only 34 of which autopsy was done. The great majority of reported cases are in the German literature, only 15 having appeared in the English language. There is divergence of opinion concerning the etiology of the disease, especially with regard to the association with tuberculosis, sarcoidosis, and cystitis follicularis, and there are wide gaps in our knowledge of the pathogenesis of the disease.

Kimla [3] and Blum [4] have noted the presence of severe tuberculosis in 46 and 50%, respectively, of cases of malakoplakia. Kimla's Case 1 and a case reported by Wildbolz,[5] neither included in our collected cases, were examples of a plaque-like cystitis, grossly like malakoplakia, which contained Michaelis-Gutmann bodies and malakoplakia cells, but which also contained tubercle bacilli and epithelioid cells. Kimla believed that malakoplakia was a special variety of tuberculosis. With two exceptions, reported cases which we can accept as instances of malakoplakia contained no tubercle bacilli, and none included Langhans' giant cells or epithelioid granulomata. Blum [4] and Pappenheimer [6] thought that the frequently associated tuberculosis was just coincidental in cases of malakoplakia. Recent authors have generally ignored this association.

From the Mallory Institute of Pathology, Boston City Hospital.

1. von Hansemann: Über Malakoplakie der Harnblase, Arch. path. Anat. **173**:302, 1903.

2. Michaelis, L., and Gutmann, C.: Über Einschlüsse in Blasentumoren, Ztschr. klin. Med. **47**:208, 1902.

3. Kimla, R.: Von Hansemann's Malakoplakia Vesicae urinariae und ihre Beziehungen zur plaqueförmigen Tuberkulose der Harnblase, Arch. path. Anat. **184**:469, 1906.

4. Blum, V.: Die Malakoplakia vesicae, Ztschr. Urol. **12**:401, 1918.

5. Wildbolz, H.: Plaqueförmige tuberculöse Cystitis unter dem Bilde der Malakoplakie vesicae, Ztschr. Urol. **1**:322, 1907.

6. Pappenheimer, A. M.: Malakoplakia of the Urinary Bladder: Report of 2 Cases, Proc. New York Path. Soc. **6**:65, 1906-1907.

Minelli [7] and others [8] have expressed the belief that malakoplakia is a granulomatous disease of the bladder with origin in preexisting lymphoid tissue. Gierke,[9] Pappenheimer,[8] and Wells [10] believed that the lesion was a reaction to urine which entered the bladder wall because of a loss of epithelial covering. Loele [11] suggested that there might be an increased permeability of the bladder mucosa. Landsteiner and Stoerk [12] and others [13] reiterated the belief that the disease is a chronic inflammatory lesion of the bladder. Boonstra and Bates [14] stated the belief that the disease is simply a response to nonspecific irritation.

Redewill [15] reported two cases associated with Boeck's sarcoid and considered that the bladder lesions in his cases were sarcoid lesions. French and Mason [16] also described a case associated with sarcoidosis, but stated that the malakoplakia represented a variety of chronic cystitis. Dickson, Gray, and Kidd [17] believed that they could identify monilia in malakoplakic lesions, an unconfirmed observation. McDonald and Sewell [18] suggested that the lesion represented inflammation of adrenal rests. Although Michaelis and Gutmann [2] diagnosed their cases as benign tumor, a neoplastic etiology has not otherwise been proposed.[1]

There has been much discussion about the nature of the predominant cell. But the consensus is that it is a macrophage of connective tissue origin.[12]

The characteristic Michaelis-Gutmann bodies have been produced experimentally by incubating blood, broth, urine, and colon bacilli.[1] These bodies are not specific for malakoplakia, since Wells [10] found them in three cases of banal cystitis. The presence of iron and calcium in these bodies has been much commented upon, but Bunting [19] has emphasized the association of iron and calcium in interstitial deposits of various types throughout the body.

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7. Minelli, S.: Über die Malakoplakia der Harnblase (Hansemann), Arch. path. Anat. **184**:157, 1906.

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11. Loele, W.: Ein Beitrag zur sog. Malakoplakie der Harnblase, Beitr. path. Anat. **48**:205, 1910.

12. Landsteiner, K., and Stoerk, O.: Über eine eigenartige Form chronischer cystitis (v. Hansemann's "Malakoplakie"), Beitr. path. Anat. **36**:131, 1904.

13. Folsom, A. I.: Malakoplakia of the Bladder, J. A. M. A. **73**:1112, 1919.

14. Boonstra, C. E., and Bates, P. L.: Malacoplakia of the Urinary Bladder: Report of a Case, U. S. Armed Forces M. J. **1**:1029, 1950.

15. Redewill, F. H.: Malakoplakia of the Urinary Bladder and Generalized Sarcoidosis: Striking Similarity of Their Pathology, Etiology, Gross Appearance and Methods of Treatment, J. Urol. **49**:401, 1943.

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18. McDonald, S., and Sewell, W. T.: Malakoplakia of the Bladder and Kidneys, J. Path. & Bact. **18**:306, 1913.

19. Bunting, H.: Histochemical Analysis of Pathological Mineral Deposits at Various Sites, A. M. A. Arch. Path. **52**:458, 1951.

There has been no acceptable explanation for the great preponderance of this lesion in females. Hagemann[20] has suggested that a "trophic factor" may be operative in females.

Clinically the disease may be symptomless or may cause manifestations of cystitis. It is self-limited and can be cured by fulguration.[13] In one case the lesion was thought to have caused blockage of the ureters, with secondary renal infection.[21] However, the disease is of importance largely as a diagnostic entity to be differentiated from tumor and as a distinctive pathologic lesion with interesting associations.

It is the purpose of this report to present four new cases of malakoplakia in which autopsy was done; in three the condition was associated with tuberculosis and in one with megacolon, megacholedochus, megaintestine and megaureter. Also two cases are being described to illustrate significant similarities among malakoplakia, banal cystitis, and cystitis follicularis. In one of these cases there was an associated sarcoid-like lesion of regional lymph nodes. The distinct lack of transition between cystitis follicularis and malakoplakia will be emphasized. From an analysis of these cases and the cases in the literature will be developed the proposition that this disease is a particular reaction to inflammation, modified by associated malnutrition and a monocyte trophism, the latter possibly of hormonal causation.

REPORT OF CASES

CASE 1.—R. T., a 41-year-old Syrian-American woman had suffered intermittently since the age of 33 from dysphagia, vomiting, substernal pain, anorexia, and weight loss. A diagnosis of achalasia of the esophagus was made. Medical antispasmodic therapy, bouginage, and then esophagoplasty at the age of 39, all gave only transient relief. Nine months after esophagoplasty she was hospitalized because of a recurrence of severe symptoms. She had in eight years lost 64 lb. (29 kg.) from an original body weight of 132 lb. (59.9 kg.). At her last admission she also gave a three week history of diurnal frequency of urination, nocturia (two times), burning on and after urination, constipation, and bowel incontinence. Her past history included a normal pregnancy and delivery at the age of 20, a hysterectomy for benign tumor at the age of 30, and morphine dependence at the age of 36. Examination one week before death revealed marked cachexia, white oral mucosal spots, a large liver, atrophic female genitalia, and a swollen, tender, warm, red left leg. There was moderate anemia. The hemogram revealed a red cell count of 2,000,000 per cubic millimeter, hemoglobin level of 6.15 gm. per 100 cc., white cell count of 10,600, with 78% polymorphonuclears, 21% lymphocytes, and 1% stab forms. The urine was neutral in reaction, had a specific gravity of 1.013 and gave negative reactions for albumin, sugar, and acetone. The urine sediment contained 20 to 50 white blood cells, including clumps, per high power field, 0 to 30 red blood cells, and many bacilli. The blood urea nitrogen was 17 mg. per 100 cc. The carbon dioxide-combining power was 14 mEq. per liter. Clinical diagnoses were anorexia nervosa, thrombophlebitis of the left leg, and postoperative achalasia of the esophagus. Hydration, penicillin, diethylstilbestrol, and rest gave some improvement, especially of the urinary burning, but the patient refused to eat or to permit parenteral alimentation. She died after a brief period of stupor.

Autopsy diagnoses were (1) cachexia with serous atrophy of fat, dehydration, pallor of the viscera, and lipofucsin accumulation in the liver, findings consistent with anorexia nervosa; (2) megaesophagus with remote esophagoplasty; megacolon and megaintestine, with parasympathetic ganglion cell deficiency; megacholedochus; slight megaloureter and hydronephrosis; (3) marked malakoplakia of the urinary bladder and the ureters; (4) slight pyelitis and ureteritis cystica; (5) slight chronic focal pyelonephritis; (6) slight apical parenchymal fibrosis of both lungs, acid-fast stains negative; (7) emphysema and focal fibrosis of the lungs; (8) slight arteri-

20. Hagemann, E.: Kasuisticher Beitrag zur sogenannten Malakoplakie der Harnblase, Ztschr. Urol. **44**:9, 1951.

21. Morison, J. E.: Malakoplakia of the Urinary Bladder, J. Path. & Bact. **56**:67, 1944.

osclerosis of the aorta and degenerative thickening of the mitral valve; (9) thrombosis of the left femoral and iliac veins with pulmonary embolus in the inferior division of the right pulmonary artery, weeks old.

The bladder and ureters were studded with gray-yellow, well-demarcated, 0.2 by 0.1 cm. to 2.2 by 1.4 cm. plaques and nodules, elevated 0.1 to 0.3 cm. and composed of yellow-gray and yellow-red soft tissue. Many nodules had hyperemic halos and central umbilications (Fig. 1). These plaques sharply spared the bladder trigone, which was hyperemic. There was no evidence of ureteral obstruction by the plaques or by any other gross lesion. The bladder measured 12 cm. in length and 15 cm. in circumference and contained 100 cc. of cloudy, gray urine from which Escherichia coli was grown in pure culture. There were four 0.1 to 0.4 cm., fluid-filled, mucosal cysts in the right renal pelvis and three similar cysts in the right ureter. The ureters were both slightly dilated. The kidneys weighed 90 and 100 gm. and contained several shallow,

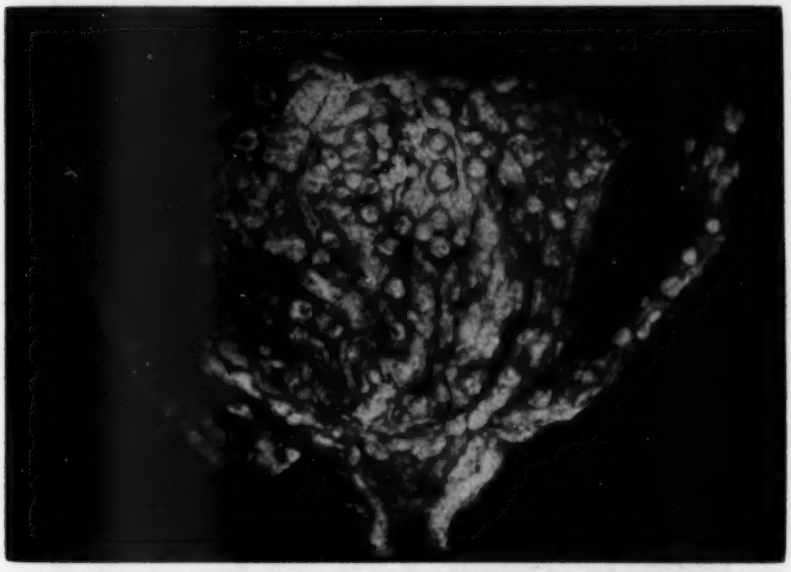

Fig. 1 (Case 1).—Bladder and ureters showing great numbers of "soft plaques"; × ⅝.

dark-based, broad scars. The adrenal cortices were thin and gray-yellow. Other organs were not remarkable.

Microscopically, the bladder mucosa was elevated and focally ulcerated over poorly demarcated submucosal masses composed of large, loosely aggregated, polyhedral and ameboid-shaped cells with small, oval or round, coarsely vesicular nuclei and much eosinophilic cytoplasm (Fig. 2*A*). Many of these cells contained small lipid vacuoles, phagocytosed red blood cells, polymorphonuclear leucocytes, bacteria, and small, light eosinophilic, solid or concentrically laminated, round bodies (Fig. 2*B*). Some of these bodies were extracellular. They were generally more numerous in the deep portions of the nodules. These bodies stained faintly for ferric iron by Perls's test, gray with Foot's silver, light yellow with resorcin-Van Gieson stain, tan with phosphotungstic acid hematoxylin, purple with Shiff's

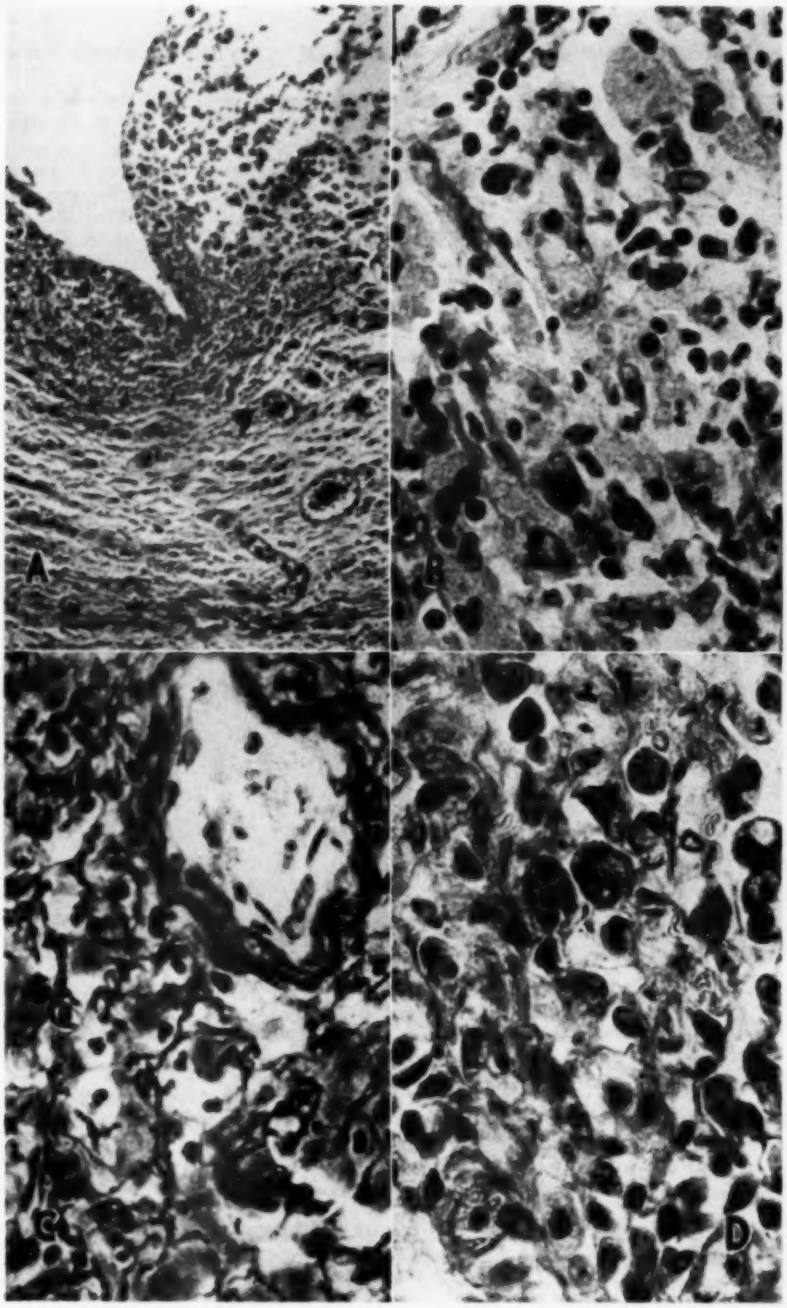

Fig. 2 (Case 1).—*A*, section through the center of one nodule and the periphery of another. Polychrome methylene blue stain; × 100. *B*, characteristic large phagocytic cells, densely stained Michaelis-Gutmann bodies, and bacteria (at bottom center of field). McCallum-Goodpasture stain; × 800. *C*, large amounts of reticulum separating individual cells of a portion of a malakoplakic nodule. Michaelis-Gutmann bodies with faintly stained laminations are also shown. Foot's reticulum stain; × 800. *D* (Case 5), macrophage accumulation in the submucosa of a common cystitis with features of malakoplakia. Polychrome methylene blue stain; × 800.

periodic acid leucofucsin and deep purple with McCallum-Goodpasture's stain. They did not stain for phosphate with van Kossa's method after 14 months' fixation in nonbuffered formalin, and they were Gram- and acid-fast-negative. There were many coliform bacilli, both outside and within cells, and a moderate number of neutrophiles, plasma cells, and lymphocytes were scattered in the nodules. There was a meager stroma of small bands of collagen, a few fibroblasts, many radially arranged capillaries, and many reticulum fibers. These reticulum fibers coursed between radial groups of cells and in many areas demarcated individual cells (Fig. 2*C*). Scattered lymphocytes, neutrophiles, plasma cells, and occasional macrophages extended into the bladder muscularis and beneath the mucosa between nodules. There were a few small, compact clusters of lymphocytes deep in the nodules, but no evidence of true lymphoid aggregates or transition from such organoid collections could be found. The changes in the ureteral nodules were identical. Acid-fast bacilli could not be stained in the lesions.

Comment.—The association of an extreme degree of typical malakoplakia with the "mega syndrome" and with great emaciation seems to be significant. Esch. coli organisms were cultured and stained in large numbers from the bladder lesion. The only possible evidence of granulomatous disease outside of the bladder was the small amount of pulmonary apical fibrosis, possibly representing healed minimal tuberculosis.

CASE 2.—M. N., a 44-year-old woman, was hospitalized in a moribund state because of extensive bilateral pulmonary tuberculosis with cavitation. There was no history of urinary symptoms, and the urine was free of blood or pus on one examination.

Diagnoses at autopsy included (1) fibrocaseous pulmonary tuberculosis of all lobes, with cavitation and bronchiogenic spread; (2) organizing fibrinous pleuritis and pneumococcic bronchopneumonia of the upper lobe of the left lung; (3) marked emaciation; (4) malakoplakia of the urinary bladder; (5) slight focal healed pyelonephritis; (6) thrombophlebitis of the left femoral and iliac veins.

The microscopic appearance of the bladder closely resembled that in Case 1. Bacilli were stained in the bladder lesions, but cultures were not made of the urine. Acid-fast stains of the bladder lesions were negative.

Comment.—This patient died of extensive pulmonary tuberculosis without extrathoracic dissemination. She was markedly emaciated. The malakoplakia was characteristic and contained many coliform bacilli.

CASE 3.—F. M., a 74-year-old woman, was transferred from a nursing home because of a senile psychosis. There was a 10-month history of dyspnea, chest pain, fever, and cough productive of thick sputum. No history of urinary symptoms could be elicited. Positive physical findings included disorientation, emaciation and dehydration, a red tongue, dullness at the base of the right lung, scattered moist lung rales, and edema. There was a moderate hypochromic anemia. The urine contained a moderate amount of albumin and occasional white blood cells.

Necropsy diagnoses included (1) fibrocaseous pulmonary tuberculosis with pleuritis, right upper lobe; (2) tuberculous peritonitis, acute, with ascites; (3) atelectasis of both lower lobes of lungs; (4) hydrothorax; (5) pulmonary congestion and edema; (6) acute pneumococcal otitis media; (7) thrombophlebitis of the left leg; (8) malakoplakia of the urinary bladder.

The lesions of the bladder were of moderate severity and of characteristic appearance, including the usual preponderance of macrophages. There were intracellular and extracellular Michaelis-Gutmann bodies. The urine was not cultured,

but masses of coliform bacilli and clusters of cocci were found in superficial portions of the bladder lesions. No acid-fast organisms could be stained in the lesions.

Comment.—This case closely resembles Case 2.

CASE 4.—W. D., a 46-year-old white woman, died of clinically obvious pulmonary tuberculosis of several years' duration. Other clinical data are not available.

Autopsy diagnoses were (1) pulmonary tuberculosis with cavitation, bilateral; (2) tuberculous laryngitis; (3) tuberculous ulcerations of the ileum, appendix, and colon; (4) uterine polyp; (5) emaciation, and (6) malakoplakia of the urinary bladder.

The malakoplakia grossly was characteristic and of great severity, similar to that in Case 1, although it did not involve the ureters. Microscopically there were large numbers of Michaelis-Gutmann bodies, moderate numbers of Gram-negative intracellular plump rods and the characteristic numerically predominant macrophages. No acid-fast bacilli could be stained. Cultures were not made of the urine or bladder.

Comment.—This case almost duplicates Case 2.

CASE 5.—*Cystitis with macrophage preponderance and giant cell granuloma of regional nodes.*

A 68-year-old white man died of septicemia from staphylococcal bacterial endocarditis. He had a 9-year history of clinical cystitis, apparently secondary to prostatic hypertrophy. The bladder at autopsy grossly represented chronic cystitis with dullness and areas of hyperemia. Microscopically it manifested a moderately dense, diffuse infiltrate of phagocytes and admixed white and red blood cells (Fig. 2*D*). No Michaelis-Gutmann bodies were seen. The reticulum pattern was like that of Case 1. There were great numbers of cocci upon the epithelium and a very few in cells and extracellularly deep in the lesions.

A few giant cell granulomatous lesions without caseous necrosis were found in regional lymph nodes. There was no evidence of tuberculosis.

The absence of gross plaques and of Michaelis-Gutmann bodies negates the actual diagnosis of malakoplakia.

CASE 6.—*Cystitis follicularis with macrophage reaction.*

A 56-year-old woman died of cerebral embolus with no evidence of tuberculosis. There was a grossly characteristic follicular cystitis and ureteritis. The centers of many of the lymphoid germinal centers in the bladder contained phagocytic cells similar to those which make up the lesion of malakoplakia. There were no apparent Michaelis-Gutmann bodies. The bladder lesion contained many cocci.

There are distinct differences between the conditions observed in this case and malakoplakia: Lymphocytes remained the predominant cell; the collections were organoid and macrophages were present in the germinal centers only; no Michaelis-Gutmann bodies were visible; the evidences of inflammation were sharply localized to the lymphoid follicle centers, and the case did not manifest the characteristic gross plaques and nodules of malakoplakia.

RÉSUMÉ OF CASE MATERIAL

These four reported cases of malakoplakia were identified in 25,030 autopsies, a 0.016% incidence. Since the disease, although self-limited and nonfatal, is relatively often seen in moribund persons, this autopsy incidence seems to be a valid index of the extreme rarity of malakoplakia.

All four of our patients were markedly cachectic. It is impossible to determine the incidence of malnutrition in previously reported cases, but it is presumed that most of the patients with severe tuberculosis were poorly nourished.

In three of our four cases there was severe tuberculosis elsewhere in the body. In one case the tuberculosis was confined to the chest; in two it included abdominal organs. This 75% incidence of severe tuberculosis in our cases of malakoplakia contrasts with a 9% incidence of severe tuberculosis in the total number of autopsies studied. This is a closer association than that in the literature, which includes 13 instances of far-advanced, active tuberculosis, representing 37 per cent of the cases in which the presence or absence of tuberculosis was commented upon. Our patients did not have tuberculosis of the bladder. In addition to the absence of apparent acid-fast bacilli, there was no true granulomatous lesion in the bladder, although the body was capable of forming typical tubercles, as evidenced in other organs.

Sarcoidosis was diagnosed in three of the previously reported cases of malakoplakia. A nonspecific sarcoid-like reaction was found in regional lymph nodes in our Case 5, in which there was a bladder lesion intermediate between cystitis and malakoplakia. As is true with tuberculosis, on morphologic evidence the bladder lesion is not sarcoid.

In all our four cases, and in 76 per cent of the previously reported cases which mention the search for organisms, coliform bacilli were stained in or cultured from lesions. In none of our four cases but in 21 previously described cases there was an associated, significant upper urinary tract inflammation. In our Case 1, in which there was an atonic megaureter, and presumably in most of the reported cases with associated urinary tract infection, there was urinary stasis.

All four of our patients were women. This compares with the 4:1 preponderance of the disease in females in reported cases. The youngest of our patients was 41 years old; hence all of our cases fall within the age group 40 or older, which includes 80% of the cases reported previously. It should be noted that there have been cases of malakoplakia in patients as young as 16 months.[22]

COMMENT

It is apparent that, despite the partial transition from other diseases, malakoplakia is a morphologic entity. Although it resembles chronic cystitis, the predominance of phagocytic cells, the characteristic plaque formation, and the Michaelis-Gutmann bodies together are distinct. And there are no morphologic transitions from cystitis follicularis to malakoplakia, although each may show features of the other.

Any explanation of the genesis of malakoplakia must explain (1) the presence of the type cells and Michaelis-Gutmann bodies; (2) the frequent association of the disease with coliform bacilli, but the inconstant bacteriologic findings and the rarity of the lesion compared with the ubiquity of coliform urinary tract disease; (3) the association of the disease with malnutrition and granulomatous disease, especially tuberculosis, and (4) the preponderance of the disease in females. It is not possible to postulate one etiologic agent to explain these facts.

22. Kairis, Z.: Zur Ursache der Malakoplakie der Harnblase, Arch. path Anat. **266**:788, 1927.

The characteristic cell, with its ability to produce reticulum, its great phagocytic properties, and its morphologic transitional forms from the fibroblast, is apparently a macrophage derived from the tissue histiocyte.

The Michaelis-Gutmann calcospherites are products of protein-rich, relatively stagnant, infected urine.[11] We have confirmed the production of these bodies by incubating blood-tryptose broth and Aerobacter aerogenes or Escherichia coli with neutral or acid urine from a healthy person. The appearance of these laminated, basophilic, calcium-iron-containing bodies recalls the appearances of pneumoliths, of prostatic corpora amylacea, and of the Schaumann and asteroid bodies of sarcoid.[16] None of these seems to have specific etiologic significance.

Cachexia may have two influences upon the development of the disease. Marasmus, including dehydration, is a factor potentiating urostasis. And marasmus may be associated with a mononuclear trophism. It has been shown recently that both monocytes and fibroblasts are affected by the stress reaction or cortisone.[23] The adrenals in our cases were not involved by tuberculosis, nor were they grossly remarkable except for moderate lipid depletion. Starvation and chronic inflammation, however, are both stressing stimuli. Although the direction of effect in malakoplakia is not now apparent, a relation between these causes and the apparent monocyte trophism seems probable.

We are unable to implicate a specific nutritional deficiency in relation to malakoplakia.

The similar basic mononuclear character of tuberculosis, sarcoidosis, and malakoplakia is striking. There is no direct evidence of a propensity of the body as a whole to react with a macrophage response to infection in these diseases. Nor is there any evidence of the presence of a bacterial toxic substance, speculated upon by Loele, which might cause this peculiar cellular reaction.[11]

Infection of the bladder, which is generally, but not necessarily, coliform in type, is one factor in the genesis of the disease. Infection of the upper urinary tract may be important only because of its association with coliform cystitis and urostasis. Since, however, coliform infections of the urinary tract are very frequent, while malakoplakia is rare, other factors must also be operative for the production of the disease. Although a few observers definitely report the absence of coliform bacilli from lesions in some cases, it is highly probable that the bacilli were present even in these cases in the lesion at some time in its development.[24]

An associated urostasis, whether secondary to cystitis or primary as in our Case 1, is a fourth presumptive etiologic factor. This is also a factor in the production of the Michaelis-Gutmann bodies. Actual urinary extravasation into the bladder wall is suggested by the deep locations of many of the extracellular Michaelis-Gutmann bodies.

Correlation of all of these etiologic factors supports the hypothesis that the macrophage reaction of malakoplakia is a unique, but not etiologically specific, inflammatory response. The cause of the rarity of the disease in males, despite the commonness of urostasis with benign prostatic hypertrophy, is not apparent.

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23. Tompkins, E. H.: The Response of Monocytes to Adrenal Cortical Extract, J. Lab. & Clin. Med. **39**:365, 1952. Selye, H.: Further Studies Concerning the Participation of the Adrenal Cortex in the Pathogenesis of Arthritis, Brit. M. J. **2**:1129, 1949.

24. Michaelis and Gutmann.[2] Pappenheimer.[6] Morison.[21]

SUMMARY AND CONCLUSIONS

Four new cases of malakoplakia, all associated with cachexia, one associated with the "mega syndrome," and three associated with severe active tuberculosis are reported, bringing the total of reported cases to 63. A phagocytic cell response similar to malakoplakia is also described in a case of common cystitis and in one of cystitis follicularis.

Analysis of these new cases and of the previously reported cases indicates the frequent association of the disease with urinary tract infections, especially those caused by the coliform organisms, the frequent association of the disease with tuberculosis or sarcoid, the association of the disease with malnutrition, and the relative selection of female patients.

The origin of the predominating component cells from histiocytes is indicated by their reticulum production and their phagocytic behavior. The similarity of Michaelis-Gutmann bodies to other calcospherites is indicated.

The hypothesis is presented that the disease is due to an interaction of urostasis and coliform infection, with a mononuclear trophism due to marasmus and chronic granulomatous infection.

Case Reports

GRANULAR-CELL MYOBLASTOMA OF COMMON BILE DUCT

Report of a Case with Autopsy Findings

ROBERT P. COGGINS, M.D.
ATLANTA

A GRANULAR-cell myoblastoma obstructing the common bile duct near the ampulla of Vater was found in a patient dying in hepatic insufficiency produced by obstructive biliary cirrhosis. The unusual location and the histologic aspects of the growth seem to warrant this report.

REPORT OF CASE

A 25-year-old married Negro woman was seen first at Grady Memorial Hospital on Jan. 22, 1952, at which time she was moribund. The present illness was of about one year's duration. Anorexia and a vague abdominal complaint were the major symptoms. The patient drank a considerable, but unknown, amount of alcoholic beverages.

There had been approximately 20 lb. (9.1 kg.) weight loss during this period. A slowly progressive abdominal distention had been present for about two months. Jaundice became evident at least one month prior to admission of the patient, but she continued to do her household duties and never sought medical advice. Three days prior to admission she became lethargic. Her breathing became progressively more labored. About 24 hours before coming to the hospital she became comatose.

On admission, the temperature was 100 F., pulse rate 60 beats a minute, respirations 30 to 40 a minute, and blood pressure 110/50 mm. Hg. The patient was poorly nourished and dehydrated. She was comatose, and her breathing was labored. The skin and scleras were deeply icteric. The neck veins were markedly distended. The heart sounds were not well heard owing to coarse rales audible over the entire chest. The abdomen was rotund, and a fluid wave was present, with shifting dullness. The liver was palpated 4 cm. below the right costal margin. Moderate pedal edema was present. The tendon reflexes were hyperactive.

The only laboratory data available revealed hemoglobin of 2.4 gm. per 100 cc.; a red blood cell count of 1,310,000 per cubic millimeter; a hematocrit reading of 9 mm. with an icteric plasma; a white blood cell count of 25,400 per cubic millimeter with a marked "shift to the left."

The patient was treated with nasal administration of oxygen and rapid digitalization. Intracardiac administration of epinephrine was tried, but the patient died two hours and five minutes after being admitted to the hospital.

The clinical diagnoses were Laennec's cirrhosis, portal hypertension, esophageal varicosities with hemorrhage, and congestive heart failure with terminal pulmonary edema.

Morphological Findings.—Gross Autopsy Findings: When the duodenum was opened, the ampulla of Vater appeared normal in size and location, but the common bile duct could not be probed from below. On dissection, the extrahepatic bile ducts were found to be markedly dilated and contained black tenacious bile with many 1 to 2 mm. "sand-like" black friable concretions. The distal portion of the common bile duct displayed an ill-defined yellow homogeneous and rubbery area of thickening which measured 3.5 by 0.6 cm. in its largest diameters. The mucosa overlying this area appeared intact. The terminal 1 to 2 mm. of the common bile duct before reaching the ampulla of Vater was uninvolved. The lesion extended into the adjacent pancreatic tissue, some lobules of which appeared to be compressed.

From the Departments of Pathology, Emory University School of Medicine, and Grady Memorial Hospital.

The pancreatic duct was of normal caliber and entered the duodenum 0.5 cm. distal to the ampulla of Vater. The pancreas (100 gm.) was of normal size, color, and consistency.

The gall bladder showed fibrous adhesions to the parietal peritoneum and contained about 25 cc. of black tenacious bile, but no stones were present. The wall was thickened, but the mucosa was smooth and light green in color. The cystic duct was dilated to 5 mm. in circumference. The wall was thin, and the normal spiral valves of Heister were absent. The mucosa of the cystic and of the common bile duct were stained dark green in color.

The liver (2,200 gm.) was adherent to the diaphragm by many dense fibrous adhesions. The capsule was uniformly thickened. The yellowish-green cut surface of the liver revealed marked distortion of the lobular architecture by firm narrow bands of grayish tissue. Some of the intrahepatic bile ducts were dilated, were filled with tenacious black bile, and contained 1 to 2 mm. of black friable concretions. Their mucosa was stained a dark green color. The lymph nodes around the hilus of the liver were somewhat enlarged.

Additional findings include ascites (3,900 cc.) and fibrous pleural adhesions on the left. The heart was slightly enlarged (330 gm., normal 267 ± 30 gm.) and on cut section the myocardium

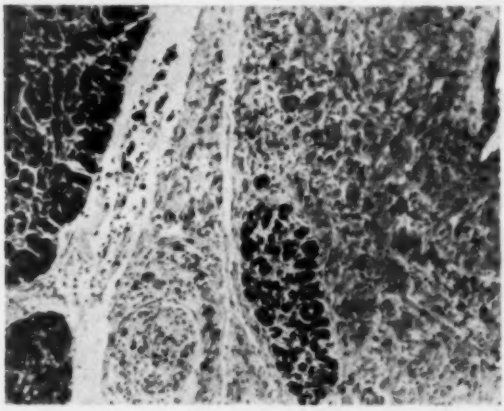

Fig. 1.—Granular-cell myoblastoma in wall of common bile duct (right) extending into pancreas. Hematoxylin and phloxine; × 95.

revealed the "thrush-breast" appearance. The lungs (left, 650 gm. and right, 750 gm.) were congested. The spleen (250 gm.) was enlarged, and the capsule was thickened. An old left tubo-ovarian abscess was found. The kidneys (left, 220 gm., and right, 210 gm.) and the adrenals (combined weight 13.5 gm.) were not remarkable. There was a small tumor (0.7 cm. in its greatest diameter) in the leptomeninges covering the left temporoparietal lobe of the brain.

Postmortem cultures yielded Escherichia coli from the heart blood and no growth from the lung.

Histologic Findings: Histologic preparations were stained routinely with hematoxylin and phloxine, while some sections were stained with phosphotungstic acid hematoxylin, Mallory's aniline blue, and Wilder's reticulum stains.

Many sections taken through the lesion of the common bile duct and the adjacent tissue revealed an ill-defined tumor. The growth occupied the wall of the common bile duct, markedly increasing its thickness. The mucosa was not altered, and some of the mucosal glands could be seen incorporated in the tumor. The tumor extended beyond the wall of the bile duct into the interstitial connective tissue of the pancreas, as well as into the loose areolar tissue near the point where the bile duct reached the pancreas (Fig. 1).

The tumor was composed of large round to spindle-shaped cells of uniform size, with small dense nuclei and abundant granular acidophilic cytoplasm. The cell boundaries were distinct. No mitoses were encountered. The cells, in general, were arranged in bundles but sometimes revealed a whorling pattern. A striking relationship of the tumor cells to nerve trunks was observed in many instances (Fig. 2). Nerve trunks were seen incorporated in some of the larger

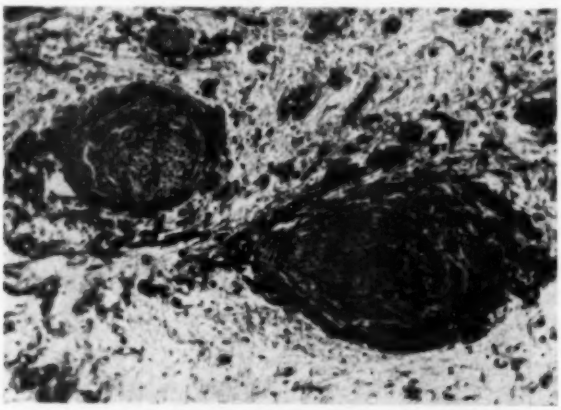

Fig. 2.—Two nerve trunks in outer wall of common bile duct. Note the relationship of the tumor cells to the nerves. Hematoxylin and phloxine; × 150.

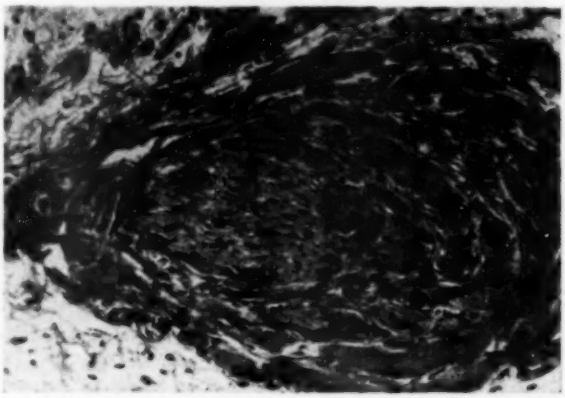

Fig. 3.—Small nerve trunk encased by tumor cells. Hematoxylin and phloxine; × 210.

tumor nodules, while at the periphery of the lesion a number of individual nerve trunks were found in which the sheath of Schwann was replaced by tumor cells (Fig. 3).

The tumor possessed an ample reticulum network and a moderate amount of collagen. At the periphery of the lesion, occasional spindle-shaped cells with the characteristics of fibroblasts, as indicated by the formation of fibroglia, could be found in which the cell body was swollen, with granular dispersions of the cytoplasm.

Despite the extension of the growth into the adjacent tissue, no evidence of new stroma formation or invasion of lymphatics or blood vessels was noted.

The liver displayed a profound distortion of its lobules, which consisted of regenerated parenchymal cells with rather incomplete rearrangement of the liver cell plates. In most lobules, a definite limiting membrane was lacking. The liver cells proper revealed a diffuse cytoplasmic vacuolation which reacted positively with fat stain. The bile canaliculi were prominent and frequently contained bile casts. Rupture of canaliculi with necrosis of the adjacent liver cells was frequently encountered. There was marked diffuse fibrosis of the portal areas associated with some chronic inflammatory infiltration. The bile ducts, and particularly their perilobular branches, revealed marked proliferation and sometimes contained bile casts.

The gall bladder revealed autolysis of the mucous membrane and fibrosis of the muscularis and perimuscular coat without significant inflammatory cell infiltration.

Additional findings were a slight degree of biliary nephrosis, pulmonary edema, and congestion. The leptomeningeal tumor was a meningioma.

Anatomical diagnoses were a granular-cell myoblastoma of the common bile duct with extension into the pancreas and pericholedochal tissues; obstructive biliary cirrhosis; chronic cholecystitis, inactive; biliary nephrosis, slight; pulmonary edema and congestion; ascites (3,900 cc.); pleural adhesions, fibrous, left; peritoneal adhesions, fibrous, and meningioma.

COMMENT

The cause of death in this patient was hepatic failure associated with obstructive biliary cirrhosis. A tumor located in the common bile duct near the ampulla of Vater produced the obstruction. Histologically the tumor was benign and met the criteria for the diagnosis of granular-cell myoblastoma. To my knowledge the occurrence of this tumor in the biliary tract has not been reported.

In 1926 Abrikossoff [1] first described the so-called granular-cell myoblastoma. It was his opinion that these lesions represented a degenerative process following injury or inflammation. As the result of further personal study and the work of others, he later revised his opinion to consider the lesion as neoplastic, originating from embryonic muscle rests.[2] The tumors were found most frequently in the tongue, skeletal muscle, and skin. Many recent authors still hold to the opinion of a myoblastic origin. In 1939, an additional hypothesis was expressed by Leroux and Delarue, who believed that the tumor cells are mesenchymal in origin and store unknown products.[3]

Crane and Tremblay, in 1945, reviewed the literature on granular-cell myoblastomas.[4] They reported the commoner sites of the tumor as the tongue, extralingual skeletal muscle, and skin. Other, less frequent, sites are bone, breast, bladder, bronchus, esophagus, and vocal cords. The occurrence of this tumor in the biliary tract does not appear to have been recorded.

Fust and Custer [5] suggested a neurogenic origin for the granular-cell myoblastoma. They based their conclusions upon the resemblance of the lesion to that seen in peripheral neurogenic tumors.

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1. Abrikossoff, A. I.: Über Myome, ausgehend von der quergestreiften willkürlichen Muskulatur, Arch. path. Anat. **260**:215-233, 1926.

2. Abrikossoff, A. I.: Weitere Untersuchungen über Myoblastenmyome, ibid. **280**:723-740, 1931.

3. Leroux, R., and Delarue, J.: Sur trois cas de tumeurs à cellules granuleuses de cavité buccale, Bull. Assoc. franç. l'étude cancer **28**:427-447, 1939.

4. Crane, A. R., and Tremblay, R. G.: Myoblastoma (Granular Cell Myoblastoma or Myoblastic Myoma), Am. J. Path. **21**:357-375, 1945.

5. Fust, J. A., and Custer, R. P.: On the Neurogenesis of So-Called Granular Cell Myoblastoma, Am. J. Clin. Path. **19**:522-535, 1949.

In 1950, Pearse [6] postulated that the granular cells are fibroblasts with degeneration. From a morphological and histochemical study he concluded that the granular appearance of the cells represents a regressive phenomenon produced by the accumulation of lipid-containing substances in the fibroblast. The lipid-containing substances may be qualitatively similar to those seen in the normal fibroblast. He proposed that the histogenesis of the lesion be indicated by the term "lipid granular-cell fibroblastoma" and indicated that the tumor may arise from perineural and endoneural fibroblasts.

The histologic findings in this tumor appear to support the concept of the fibroblastic origin of the growth and suggest that the growth arose from perineural or endoneural fibroblasts.

SUMMARY

We are presenting a case in which a granular-cell myoblastoma of the common bile duct produced obstructive biliary cirrhosis and death in hepatic failure. The histologic findings suggest that the tumor arose from perineural and endoneural fibroblasts.

6. Pearse, A. G. E.: The Histogenesis of Granular-Cell Myoblastoma (? Granular-Cell Perineural Fibroblastoma), J. Path. & Bact. **62**:351-362, 1950.

RENAL APLASIA IN SISTERS

EDWARD C. H. SCHMIDT, Ph.D., M.D.
EASTON, MD.
ARTHUR A. HARTLEY, M.D.
AND
ROBERT BOWER, M.D.
PHILADELPHIA

BILATERAL renal aplasia has generally been considered a rare congenital defect. However, Potter [1] in 1946 presented a series of 20 cases taken from reports on approximately 5,000 infants subjected to postmortem examination. In an analysis of the 135 instances of bilateral renal aplasia reported in the literature before 1937, Amolsch [2] pointed out that the sex ratio is 2.7 males to 1 female and concluded that there was no plausible explanation for the sex discrepancy. Madisson [3] reported two cases of bilateral renal agenesis occurring in nontwin male fetuses born of a 31-year-old white woman. We recently encountered two cases of renal aplasia occurring in nontwin sisters.

REPORT OF CASES

In 1948, the mother, a healthy 25-year-old white woman, was delivered of a normal full-term girl, after an uneventful antepartum course. In 1949, she was again delivered of a full-term normal girl. Both of these children are living and well. The mother was admitted to the hospital in active labor on Sept. 1, 1950, after an uneventful antepartum course. The fetus was delivered from a frank breech position. At delivery, it was noted that there was a relatively small amount of amniotic fluid. The baby was cyanotic at birth and after laryngoscopy and use of a respirator took her first breath four minutes after delivery. However, despite supportive therapy, the child became pallid and limp and died two hours after birth.

Postmortem examination revealed a white girl weighing 2,150 gm. and measuring 47 cm. from crown to heel. The lungs together weighed 14 gm. The pleural surfaces were smooth and glistening. A small quantity of thin exudate was present in the bronchi, and there were areas of atelectasis measuring a few millimeters in diameter scattered throughout both lungs. The anus was patent.

The adrenals were oval and flat, measuring 6 mm. in thickness and weighing a total of 6 gm. No kidneys were found in a careful search extending from the diaphragm to the pelvis. The renal arteries had normal orifices at the aorta and extended into the fat caudad to the adrenals. No ureters could be found at the site of the renal fossae. The bladder was represented by a slender sac, which had an internal length of 13 mm. Projecting from the base of the bladder on the left, there was a small cord-like structure which measured 13 mm. in length and 1 to 2 mm. in diameter, and which resembled a ureter. A similar structure could not be identified on the right side of the base of the bladder. A small urethral opening was present. The uterus was of normal size and shape for a newborn infant. The ovaries were small and of a brown-yellow color. The Fallopian tubes were thin and coiled.

Microscopic examination of the bladder revealed that the wall was composed of thick interlacing bundles of smooth muscle fibers and that the lining was composed of transitional epithelium, similar to that usually seen. The tube of tissue extending from the left side of the bladder was composed of bundles of smooth muscle fibers arranged about a small lumen lined

From the Department of Pathology, Hahnemann Medical College and Hospital of Philadelphia.

1. Potter, E. L.: Bilateral Renal Agenesis, J. Pediat. **29**:68-76, 1946.
2. Amolsch, A. L.: Bilateral Metanephric Agenesia, J. Urol. **38**:360-370, 1937.
3. Madisson, H.: Über das Fehlen beider Nieren, Zentralbl. allg. Path. **60**:1-8, 1934.

with transitional type epithelium. Sections of the uterus showed infantile endometrium. Sections of the lungs showed many of the alveoli to be unexpanded. Approximately one-third were expanded and apparently air-containing. Many of them were filled with red blood cells or a mixture of red-staining material and red blood cells. A few alveoli had areas of cuboidal epithelium on their walls. Sections of the adrenals revealed the fascicular layer composed of rather large cells with dense, red, granular cytoplasm, resembling fetal cortical cells.

The diagnoses were bilateral renal agenesis; absence of right ureter; hypoplastic left ureter; pulmonary hypoplasia, and liver hemopoiesis.

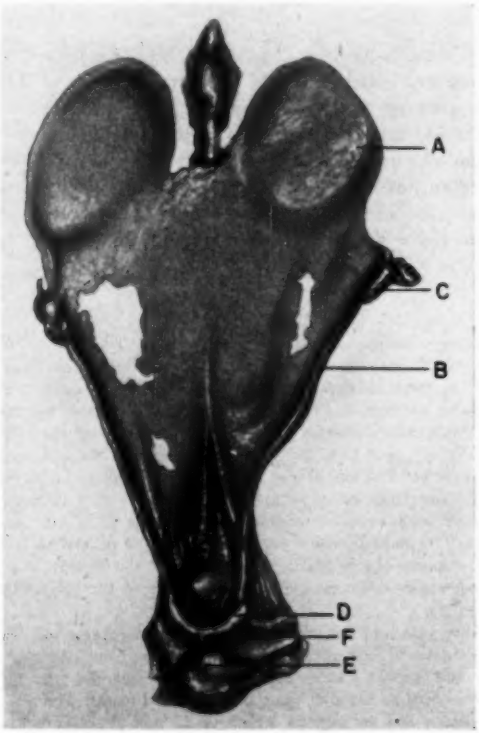

Fig. 1 (Case 2).—Anterior view of gross specimen showing discoid adrenals (*A*) and ureter-like cords (*B*) with nodules at craniad ends (*C*) and Fallopian tubes (*D*) at caudal ends. The bladder-like sac (*E*) is anterior to the fused Fallopian tubes representing the uterus (*F*).

An investigation of the family did not reveal a history of any congenital defects. The mother is an only child, and her mother, with a clear recollection of the two preceding generations, remembered no similar abnormalities. The father, aged 30, and in good health, has two sisters, both of whom produced normal children. His mother is living and well and recalls no abnormal children in the preceding two generations. It is a point of interest that each of the four grandparents of these sisters was an "only child." On the basis of this information the mother was advised that any similar abnormality was unlikely in future pregnancies.

On Jan. 11, 1952, after a normal antepartum course, the mother was delivered of a full-term girl. Again, a reduced amount of amniotic fluid was noted. The infant was cyanotic and dyspneic from birth, with tugging respirations. An abnormally flat nose with concavity of the bridge was noted. A chest x-ray revealed enlargement of the heart with pressure anteriorly on the

trachea. Despite laryngoscopy, oxygen, and supportive therapy, the infant died 10½ hours after delivery.

Postmortem examination showed a white girl weighing 3,000 gm. and measuring 46 cm. from crown to heel. The nose was concave and flattened, with spreading of the nares and a cleft in the cartilage at the tip. The mouth was small. The jaw was receding and flat across the point. The tops of the ears were flat and folded.

A large defect was present in the atrial septum of the heart beside the patent foramen ovale, which reduced the interatrial wall to a thin rim of tissue about the periphery, with a thin band crossing the center. The pleural surfaces of the lungs were smooth. The lungs weighed 21 gm., and on section, the parenchyma was dark red and contained only a little air. The anus was patent.

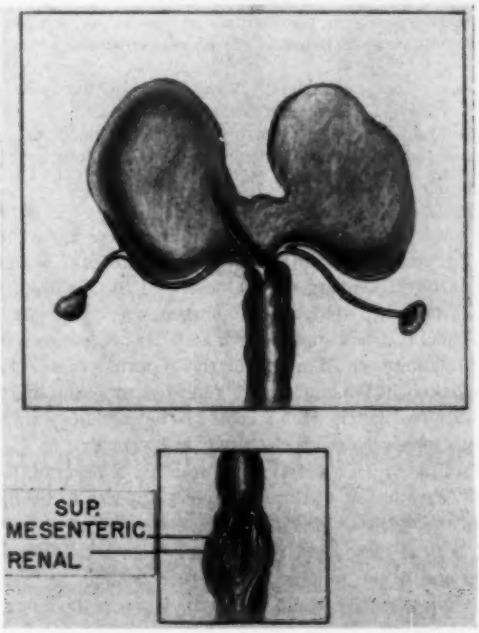

Fig. 2 (Case 2).—Posterior view of aorta, illustrating single renal artery and its branches. The lower view shows the one orifice for the renal arteries.

The adrenals were discoid in shape, measuring 4 by 3 by 0.7 cm. The adrenals were in their usual position but did not have the folded shape characteristic of this organ. Approximately 1.5 cm. below the lower edge of the adrenals, there were small oval-shaped nodules, which measured 10 by 4 by 3 mm. These nodules on section were firm and homogeneous. Extending caudad from these nodules were small bands of tissue in peritoneal folds. These bands followed the general course of the ureters, but joined the distal ends of the Fallopian tubes. Cross sections of these bands did not reveal any lumina, and they were composed of firm gray tissue.

There was an irregular nodule measuring 14 by 8 by 7 mm. replacing the uterus. At the lower end of this uterine nodule, there was a small pouch, measuring 4 mm. in diameter, which did not have an external opening. Extending from the nodular structure representing the uterus, there was a tube on each side measuring 8 mm. in length and 4 mm. in diameter, which resembled a Fallopian tube. From the distal ends of these Fallopian tubes the cord-like structure extended toward the adrenals. There was a sac-like structure anterior to the uterus measuring 9 mm. in length, which apparently represented the bladder. The lining was white, and a patent

urethra was present. No ureteral orifices could be found, nor could a connection between the bladder and the cord-like structures arising from the Fallopian tubes be established.

At the site just below the level of the superior mesenteric artery, where there are usually the orifices of two renal arteries, only one orifice could be seen. After leaving the aorta, this artery divided in two, one branch extending to the adrenal on each side.

The microscopic examination of the tubular bodies arising at the sides of the uterine nodule showed villous processes covered with epithelium characteristic of Fallopian tubes. A section through the nodules forming the main uterine body and bladder showed one cavity lined with transitional epithelium suggesting a bladder, and another lined by squamous epithelium, apparently representing a vagina. Beneath the transitional epithelium there were interlacing bands of smooth muscle. The vagina was lined with thick squamous epithelium which in some foci was 15 cells in thickness. Sections taken through the nodules at the ends of the Fallopian tubes showed distorted ovarian elements and numerous small ova.

The cord-like bands running in the folds of peritoneum, as well as the nodules at the cranial ends of the cords, were composed of smooth muscle bundles and numerous small blood vessels.

The lungs had many unexpanded alveoli. Many of the alveolar lining cells were cuboidal, and the alveoli were collapsed or contained hemorrhagic edema.

The diagnoses were bilateral renal agenesis; malformation of uterus, bladder and vagina; pulmonary hypoplasia; interatrial septal defect; hemopoiesis of liver, and facies characteristic of renal aplasia.

COMMENT

These two cases of bilateral renal agenesis in sisters apparently constitute the first instance of this condition occurring in female siblings. The only other reported case of this condition in siblings is that described by Madisson [3] in a report on bilateral renal aplasia in two nontwin male fetuses, which had one living, apparently normal, sister. That this defect should occur in sisters is further remarkable because of the marked predominance of males in the reported cases. Potter observed a strong male sex-linked factor, and in the reported series of this congenital defect males constitute approximately 70 to 80% of the patients exhibiting a tendency toward sex linkage which is exceeded only by anencephaly.

The mechanism by which this lesion is produced is obscure, but in both of our cases the lack of development of normal ureters appears to be the basic defect in the mechanism of the agenesis.

The lungs in both of these cases weighed less than anticipated in relation to the total body weight and to the weights of the other organs. In Case 1, the expected combined lung weight normally would be 44 gm., whereas the lungs actually weighed 14 gm., or 32% of normal. In the second case, the lungs weighed 21 gm. or 39% of normal. In 1946, Potter [4] described a condition occurring in the lungs in cases of renal agenesis, to which she applied the term "pulmonary hypoplasia." The weights of the lungs in the cases in her series varied from 15 to 60% of the average lung weights for normal fetuses of similar body weights. Both the weights, and to a less extent, the microscopic picture in our cases, are compatible with the diagnosis of "pulmonary hypoplasia."

SUMMARY

Two cases of bilateral renal agenesis occurring in nontwin sisters are described. The parents of these two girls also have two normal girls who are living and well. There is no known history of any congenital defect within the three previous generations in either family.

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1. Schilling, F. J.; De Natale, A.; and Mottram, F. C.: Am. J. M. Sc. 222:207 (Aug.) 1951. 2. Shapiro, S., et al.: Am. Heart J. 40:766 (Nov.) 1950. 3. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey 48:1 (Jan.) 1951.

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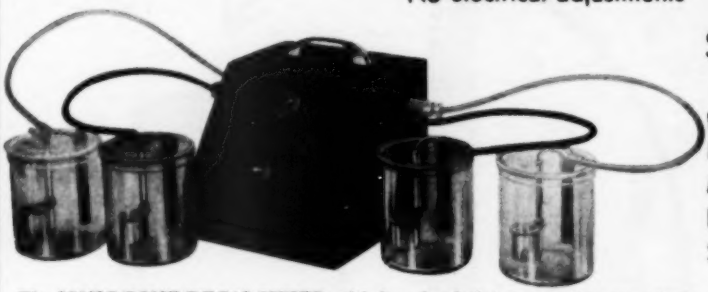

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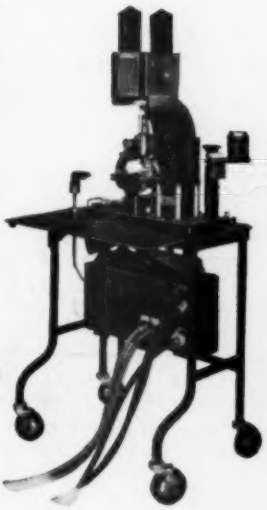

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